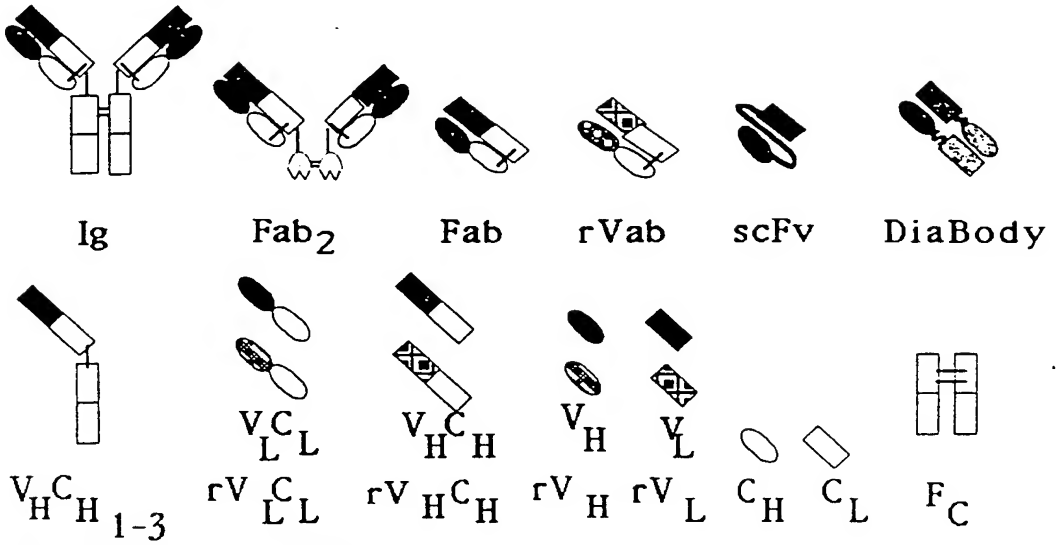
STAGE	ACTIVITY	PRODUCT
Stage I		
a.	Construct Recombinant Antibody Library	:rVab.lib
b.	Select rVab which Bind Target Specifically	:rVabTS+
c.	Isolate rVabTS+ Which Regulate Target Activity	:rVabTSA+
Stage II		
a.	Convert rVabTSA+ to Labelled Active Surface Reporters	:[*]rVabTSA+
	one for each unit domain of the active site	
b.	Use [*]rVabTSA+ in Binding Assays to Isolate	
	competing small organic molecules	:SOMERS
	one for each Reporter of a active site unit domain	:SOMERS-T_n
c.	confirm SOMER activity on Target	
	at single domain active sites	:SOMER(T_1+)
	at multiple domain active sites	
	chemically linking SOMERS for each active site domain	:SOMER(T_1-T_n) [= MULTIMER+]
Stage III		
a.	Extract Structural Information from rVabTSA+	: rVab InfoBase
b.	Create a Biological Enhanced Ensembled Pharmacophore	:BEEP-T_1
	(i.e.,structures which Regulate Target Activty)	
	one for each domain of the active site	:BEEP(T_{1-n})
c.	Find active SOMERS for Targets	
	(using Computation Screens and Synthetic Efforts)	
	with BEEP T_1 for single active domain of Target	:SOMER-T_1+
	with BEEP-T1-n for multiple active domains	:SOMER-T_{1-n}*
	and then chemically coupling SOMERS(T_1-T_n)	:MULTIMER+

FIG. 1

2A Antibody Structures:



2B Variable Region Domains:

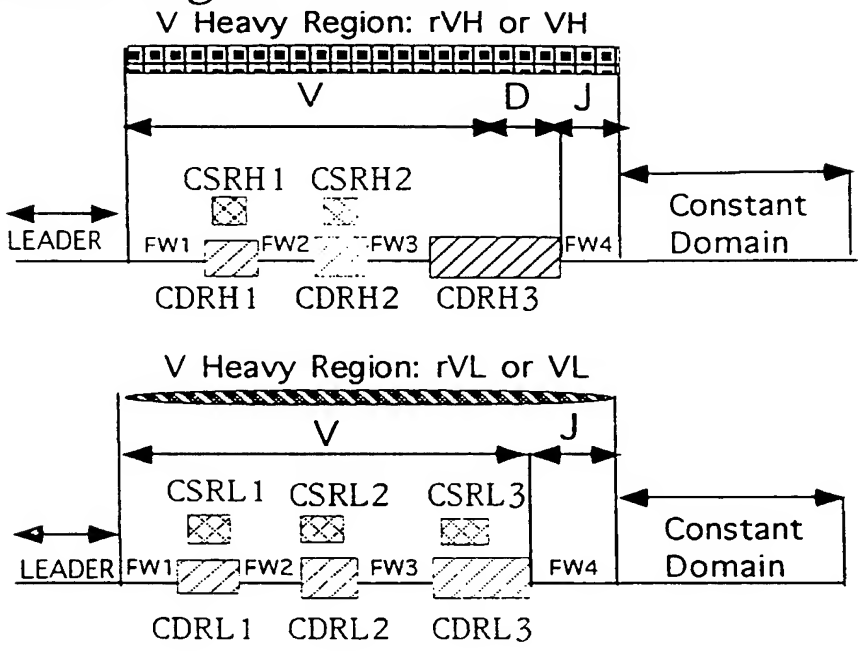


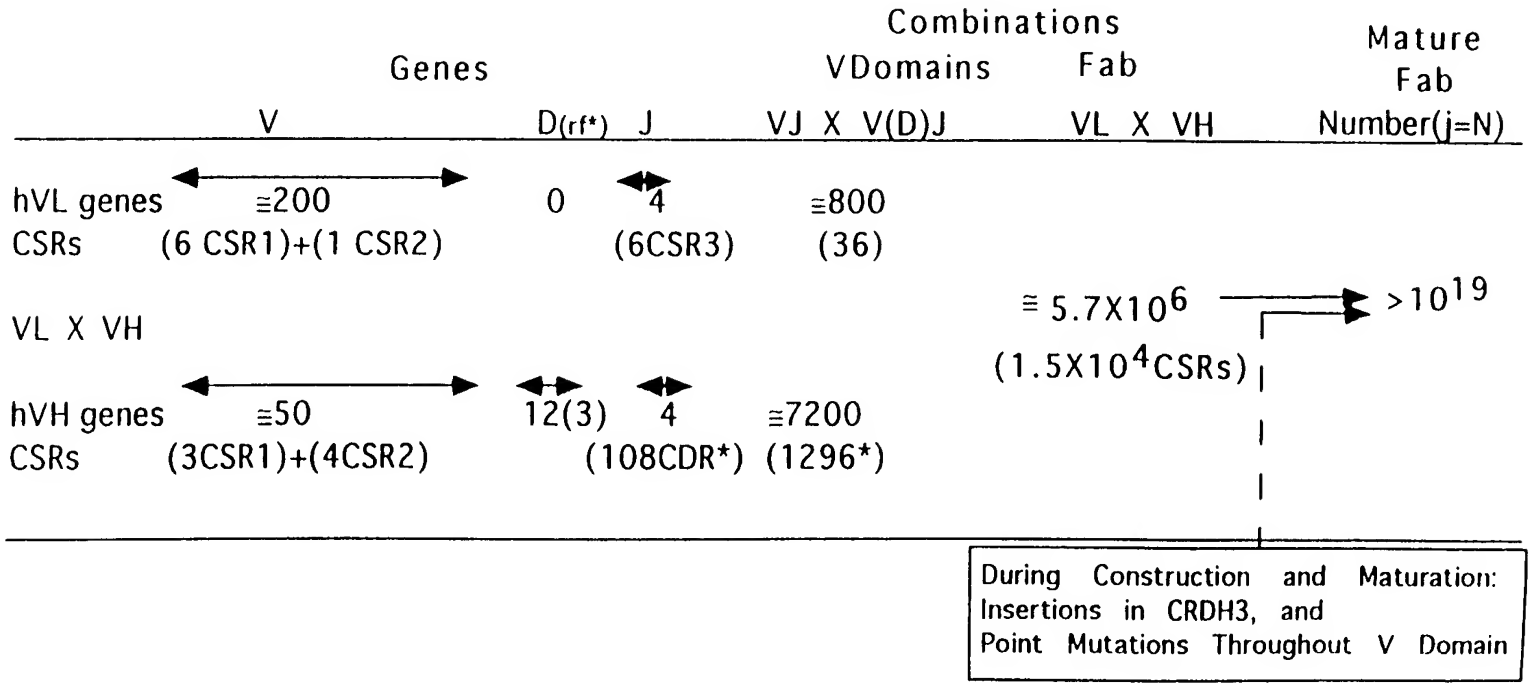
FIG. 2

Combining Site	Class	CDRH3 aaSize	CDRH3 Sequence*	Brkhvn Entry	Common Name	Type Antigen	Burried Surf.Area	VH-L IntrFce	VH-L Rotatn	Crstyl A Resoltn	AgContacts NonH3/To	Ref
cavity		5	DHG(.....)SD		NQ10/12.5	hapten	170					a,b
cavity		10	SSGNKWAM(.....)DY	1figl	26-10	hapten				2.7	6/10	a,b
cavity		6	GWP(.....)LAY	1baf	AN02	hapten				2.9	5/7	a,b
cavity				1bbd	8F5	hapten						a
cavity		10	GDYVNWPG(.....)DV	1dba;b	DB.3nat;prog	hapten	223-291	1425-1556	?	2.8		a,b
cavity					D7B2	hapten						a,
cavity		7	SYYGM(.....)DY	4fab	4-4-20	hapten	266	1375				a,b
cavity		11		1mcp	McPC603	hapten	137	1675				a
?		15	FYYGGSHLAVYY(4)FDS		R19.9	hapten				2.8	5/28	a,b
?		9		jfbj	J539	carbohydrate		1547		1.95		a
?		12	SEYYGGSYK(.....)FDY	6FAB	36-71	hapten						b
groove		10	YSSDPFYF(.....)DY	1igf;2igf	B1312;n.1	peptide		1409-1508		2.8		a,b
groove		11		1igf	B1312.n.2	peptide		1537				a
groove		11		1hil;m,n	17/9	peptide	400	1455-1545	2.3-4.2	2.0		a
groove		5		1ggi;b;c	50.1	peptide	475	1063-1175	14.8-16.3	2.8		a,b
groove		4		1igm	POT	peptide				2.3		a
groove					TE33	peptide(CTX)	503			2.9	10/46	a,b
groove		7			MCG	peptide						a
?		9		1mfb	SE115-4	saccharide						b
planar		13	SGGSYRYDGG(.....)FDY		NC10	protein(Neu)	716				9/12	c
planar		11	GEDNFGSLS(.....)DY	1nca	NC41	protein(Neu)	879				8/13	a,b
planar		8	RDYRL(.....)DY	1fdl	D1.3	protein(Lyz)	680	1453			5/15	
		a,b										
planar		5	WDG(.....)DY	3hfm	HelHel10	protein(Lyz)	774	1411			14/17	a,b
planar		7	GNYD(.....)FDG	2hfl	HyHel5	protein(Lzy)	750	1305			8/12	a,b
planar					FvD13.11	protein						a
planar		8	GLAFY(.....)FDH		E225;antiD1.3	protein(D1.3)	800					a,b
planar		9	QGTIAG(.....)IRH	7fab	NEW	myeloma		1483		2.0		a,b
?		12	DPDLILTAPS(.....)DY	8fab	HIL	myeloma				1.8		a,b
planar		8		1mam	YST9.1	carbohydrate				2.5		a
planar		8			Jel318							a,b
planar		9		2fbj	J539							a,b
planar		7		1mcb	MCG							a,b
planar		10	DQDGTA(.....)WFAY	1cbv	BV04.01;nat							
		a,b										
?					dna	DNA		1387-1404	7.5			a
?		8			Jel72							a,b
?		17		2fb4	KOL			1612		1.9		a,b

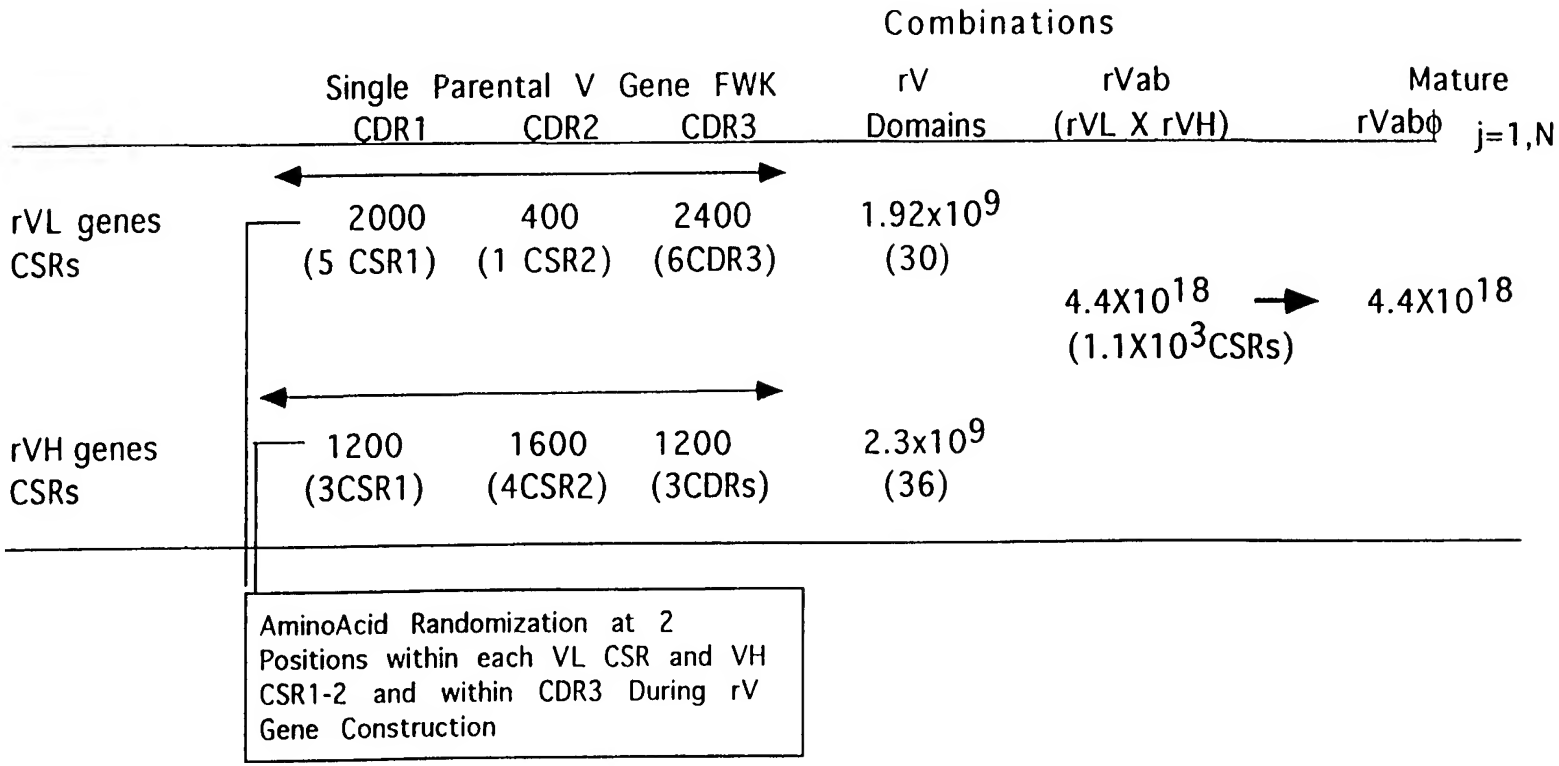
Ag:Ab Contact are v.d.W., salt bridges and hydrogen bonds. Ref a=[Webster,Henry,Rees;1994]; b = [Wu,Johnson and Kabat;1993] and c = [Malby et.al. 1994]
x/y= Ratio of aa contacts between aa of Ab not in CDRH3 (i.e., Non-CDRH3) and An to total aa contacts between Antibody(Ab) and Antigen(Ag)
* = CDRH3 Sequence given as VH aa from position 95-102 using maximum CDRH3aa size as19 and with alignment (....) as used by Wu, Johnson and Kabat [1993]

FIG. 3

4A NATURE'S IMMUNE REPERTOIRE:



4B THE rVab REPERTOIRE:



NAME	L1	L2	L3	H1	H2	H3
CDR aa						
Positions	[24-34]	[50-56]	[89-97]	[31-35]	[50-65]	[95-102]
Insertion Points	27a-f		95a-c	31ab	52a-c	100a-k
CSR						
aa Positions	[26-32]	[50-52]	[91-96]	[26-32]	[52a-55]	-
# Known	5	1	6	3	4	0
Essential aa						
in CSR	29*	-	94**,95*	26*,27*,29*	52a*,54**,55*	-
in CDR	25*,33*	-	-	-	-	-
in FW	2*,71*	48*,69*	90*	34*,94*	71*	-
Surface aa	27-33	49-53	91-96	28-33	52,-58,60	-
Burried aa	-	-	-	34	51	96-100
AA variance						
in CDR						
κ mu	30>31=28>29	50>>55>>53=51	94>92=91=93>96	35>33	50>52=53=54	95=96=100>97>98=99
κ all	28=31=34	50>>55>53=51	94>92-91=96>93	30=31=32=34		
λ all	43>31=29>28=27	50=51=52=53	95=96>94=92	-		
κ, λ all	27-31=34	50>>51=52	94>96=92>91>93	35>33>31	50>52=53=54	95=100>96>99>97=98
Nonessential						
all	28,30,31	50,51,52	94*,92,96,91,93	28,30,31,32	53,54	-
Library Diversification	28,30,31	52,50,53	91-94; 95abc	28,30,31	53,54	96-100a-k

CSR= Canonical structure; CDR = complementary determining region of high variance; FWK= framework residues
Chothia and Lesk (1987); Chothia, et.al. (1989); Kabat,E.A., et.al. (1991); Chothia, C., et.al.(1992) andTomlinson, I.M.,et al (1992).

FIG. 5

1.VL

CANONICAL STRUCTURE			CDR / CSR			CSR DIVERSITY POSITION
L1	KNOWN	2	24252627abc282930abc31abcdef323334	71	SIZE	6AA 30,32
L1.1	YES	I*	+ A*S S ---S V*+ ---- -------+ M*+		Y*	6AA 30,32
L1.2	YES	I*	+ A*S Q ---S I*+ ---+ -------+ L*+		Y*	7AA 30,31
L1.3	YES	I*	+ S*S E ---S L*+ ---+ SGNEKN+ L*+		Y*	13AA 30,31
L1.4	YES	V*	+ S*Q S ---S L*+ ---+ S-NGNT+ L*+		F*	12AA 30,31
L1.5	YES	S	+ G*S S ---S D*+ GS-+ -------+ L*+		A*	9AA 30,31
L1.6	NO	S	+ G D N ---L N + ---+ -------+ V*+		A*	7AA 30,31

CANONICAL STRUCTURE			CDR / CSR			CSR DIVERSITY POSITION
L2	KNOWN	4849	50515253545556	64	SIZE	5 0 , 5 1
L2	YES	I*+	+ + S + + + +	G*	3AA	5 0 , 5 1

CANONICAL STRUCTURE		CDR / CSR			CSR DIVERSITY POSITION
L3	KNOWN	8990919293ABCDEF94959697		SIZE	9 3 , 9 2
L3.1	YES	+ Q*+ + + -------+ P*+ +		6AA	9 3 , 9 2
L3.2	YES	+ Q*+ + + -------P*+ + +		6AA	9 3 , 9 2
L3.3	YES	+ Q*+ + + -------+ P*- +		5AA	9 3 , 9 2
L3.4	YES	+ Q*+ + + -------P⁻P⁻+ +		6AA	9 3 , 9 2
L3.5	YES	+ Q*+ + + ++-----+ + + +		8AA	9 3 , 9 2
L3.6	YES	+ Q*+ + + +------+ + + +		7AA	9 3 , 9 2

2.VH

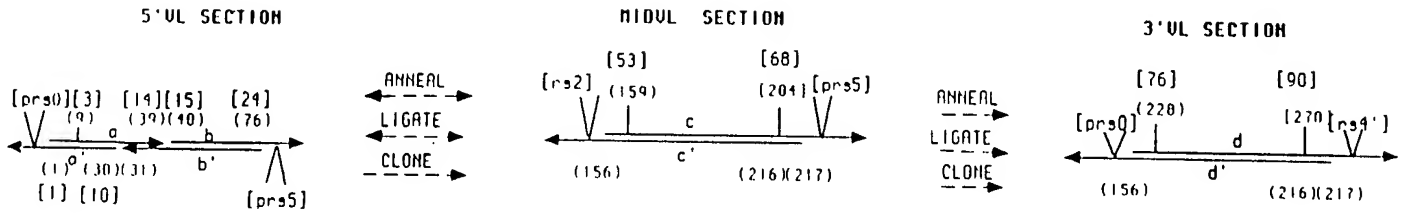
CANONICAL STRUCTURE		CDR / CSR			CSR DIVERSITY POSITION
H1	KNOWN	2425262728293031ab323334	94	SIZE	3 1 , 2 8
H1.1	YES	A*+ G*F*+ F*+ + --+ + M*	R*	7AA	3 1 , 2 8
H1.2	YES	A*+ G*S*+ F*+ + --+ + W*	R*	7AA	3 1 , 2 8
H1.3	YES	A*+ G*Y*+ F*+ + +-+ + W*	R*	7AA	3 1 , 2 8
H1.4	NO	A*+ G*F*+ F*+ + +++ + M*	R*	7AA	3 1 , 2 8

CANONICAL STRUCTURE		CDR / CSR			CSR DIVERSITY POSITION
H2	KNOWN	505152a bc535455565758596061626364 65 71		SIZE	5 3 , 5 4
H2.1	YES	+ + + - --+ + G*+ + + + + + + + + + V*		4AA	5 3 , 5 4
H2.2	YES	+ + + P*--+ + G*+ + + + + + + + + + A*		5AA	5 3 , 5 4
H2.3	YES	+ + + P*--+ G*F + + + + + + + + + + A*		5AA	5 2 , 5 3
H2.4	YES	+ + + N*KG+ K*Y*+ + + + + + + + + + A*		7AA	5 2 , 5 3

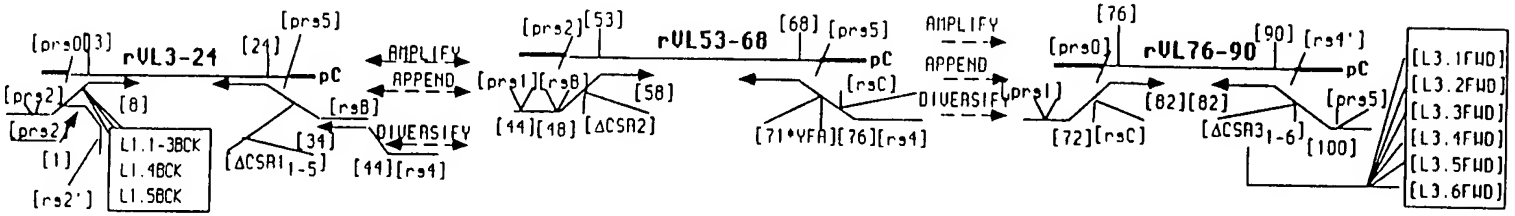
* = NONRANDOM,CRITICAL CSR AA RESIDUE(S);
\+ = PARENTAL (or REPLACEMENT) AA RESIDUE NONESSENTIAL TO CSR;
\- = AA RESIDUE NOT PRESENT; P⁻ = ANY AA RESIDUE BUT PROLINE;
DIVERSITY POSITION = POSITION HAVING RANDOMIZED AA IN LIBRARY

FIG. 6

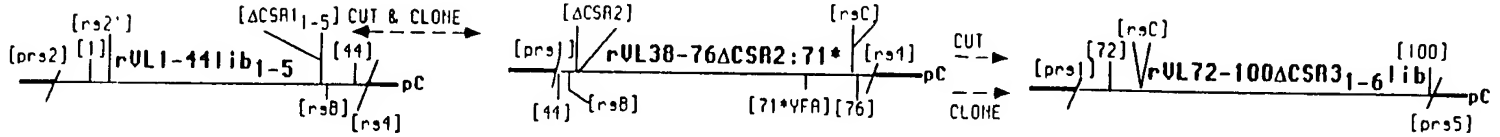
7A


5'VL SECTION
MIDVL SECTION
3'VL SECTION
ANNEAL
LIGATE
CLONE
[prs0][3] [14][15] [24]
(9) (39)(40) (76)
a
b
(1) (30)(31)
a'
b'
[1] [10]
[prs5]
[53] [68]
[prs2] (159) (204) [prs5]
c
c'
(156) (216)(217)
[76] [90]
(220) [270] [prs4']
d
d'
(156) (216)(217)

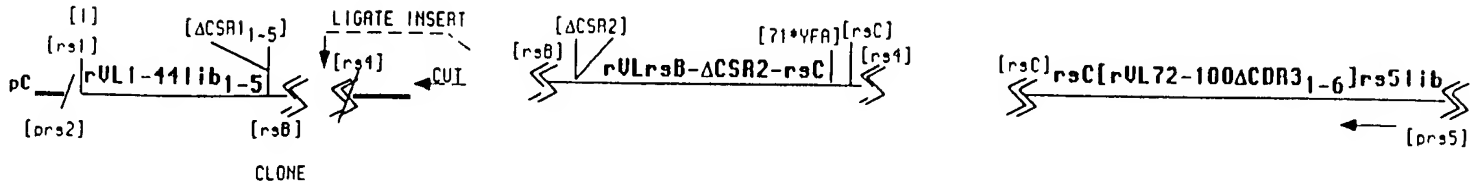
7B


[prs0][3] rVL3-24 [24] [prs5]
pC
[prs2] [8]
[prs2] [1]
[rsB]
[34]
DIVERSIFY
[44][rs4]
[rs2']
L1.1-3BCK
L1.4BCK
L1.5BCK
[ΔCSR1 1-5]
AMPLIFY
APPEND
[prs2][53] rVL53-68 [68] [prs5]
[prs1][rsB] [58]
[44][48] [ΔCSR2]
[rsC]
[71*YFA][76][rs4]
[76]
[prs0] rVL76-90 [90] [rs4']
[prs1]
[82][82]
[72][rsC]
[ΔCSR3 1-6]
[prs5]
[100]
[L3.1FWD]
[L3.2FWD]
[L3.3FWD]
[L3.4FWD]
[L3.5FWD]
[L3.6FWD]

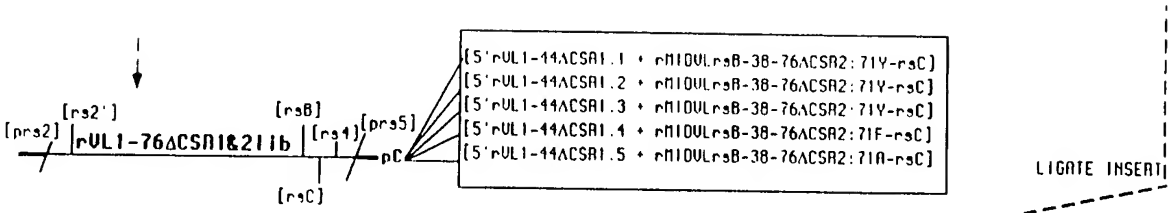
7C


[rs2'] [ΔCSR1 1-5] CUT & CLONE
[prs2] [1] rVL1-44lib 1-5 [44]
pC
[rsB] [rs4]
[ΔCSR2] [rsC]
[prs] rVL38-76ΔCSR2:71* [rs4]
pC
[44] [rsB]
[71*YFA][76]
CUT
CLONE
[rsC]
[prs] [72] rVL72-100ΔCSR3 1-6 lib [100]
pC
[prs5]

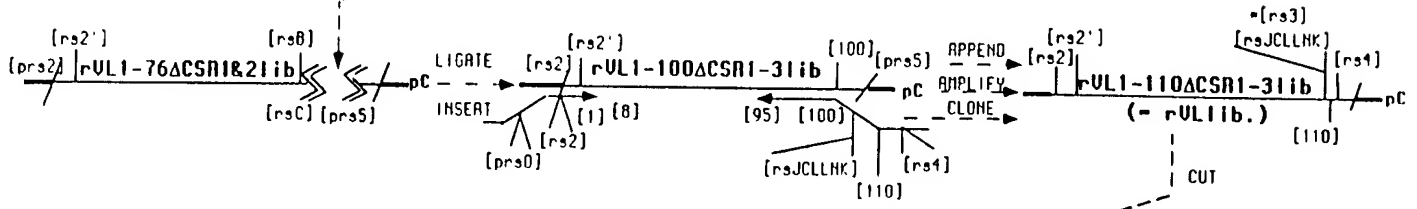
7D


[1]
[rs1]
[ΔCSR1 1-5]
LIGATE INSERT
pC
rVL1-44lib 1-5
[rs4]
CUI
[prs2]
[rsB]
CLONE
[ΔCSR2]
[71*YFA][rsC]
[rsB]
rVLrsB-ΔCSR2-rsC
[rs4]
[rsC] rsC[rVL72-100ΔCDR3 1-6]rs5lib
[prs5]

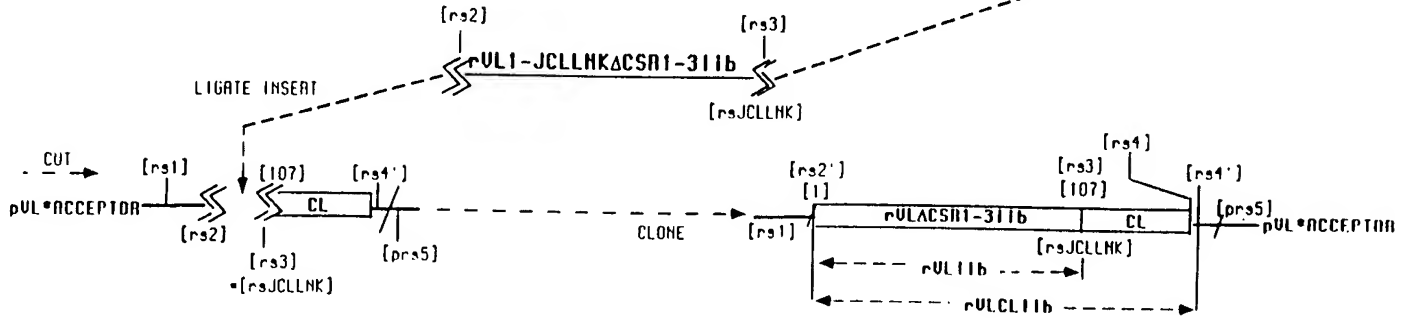
7E

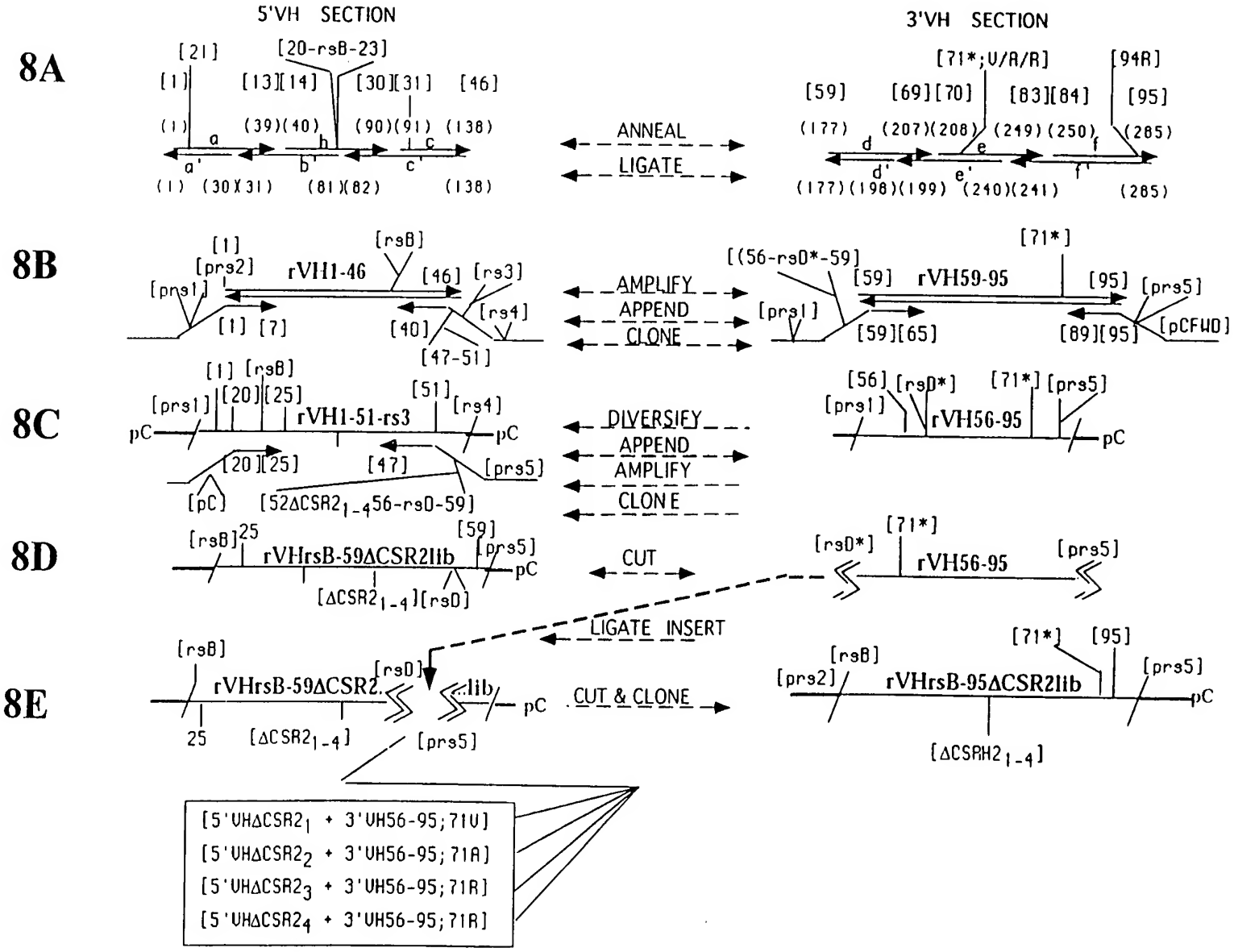

[rs2']
[prs2]
rVL1-76ΔCSR1&2 1b
[rsB]
[rs4]
[prs5]
pC
[rsC]
[5'rVL1-44ΔCSR1.1 + rMIOVLrsB-38-76ΔCSR2:71Y-rsC]
[5'rVL1-44ΔCSR1.2 + rMIOVLrsB-38-76ΔCSR2:71Y-rsC]
[5'rVL1-44ΔCSR1.3 + rMIOVLrsB-38-76ΔCSR2:71Y-rsC]
[5'rVL1-44ΔCSR1.4 + rMIOVLrsB-38-76ΔCSR2:71F-rsC]
[5'rVL1-44ΔCSR1.5 + rMIOVLrsB-38-76ΔCSR2:71A-rsC]
LIGATE INSERT

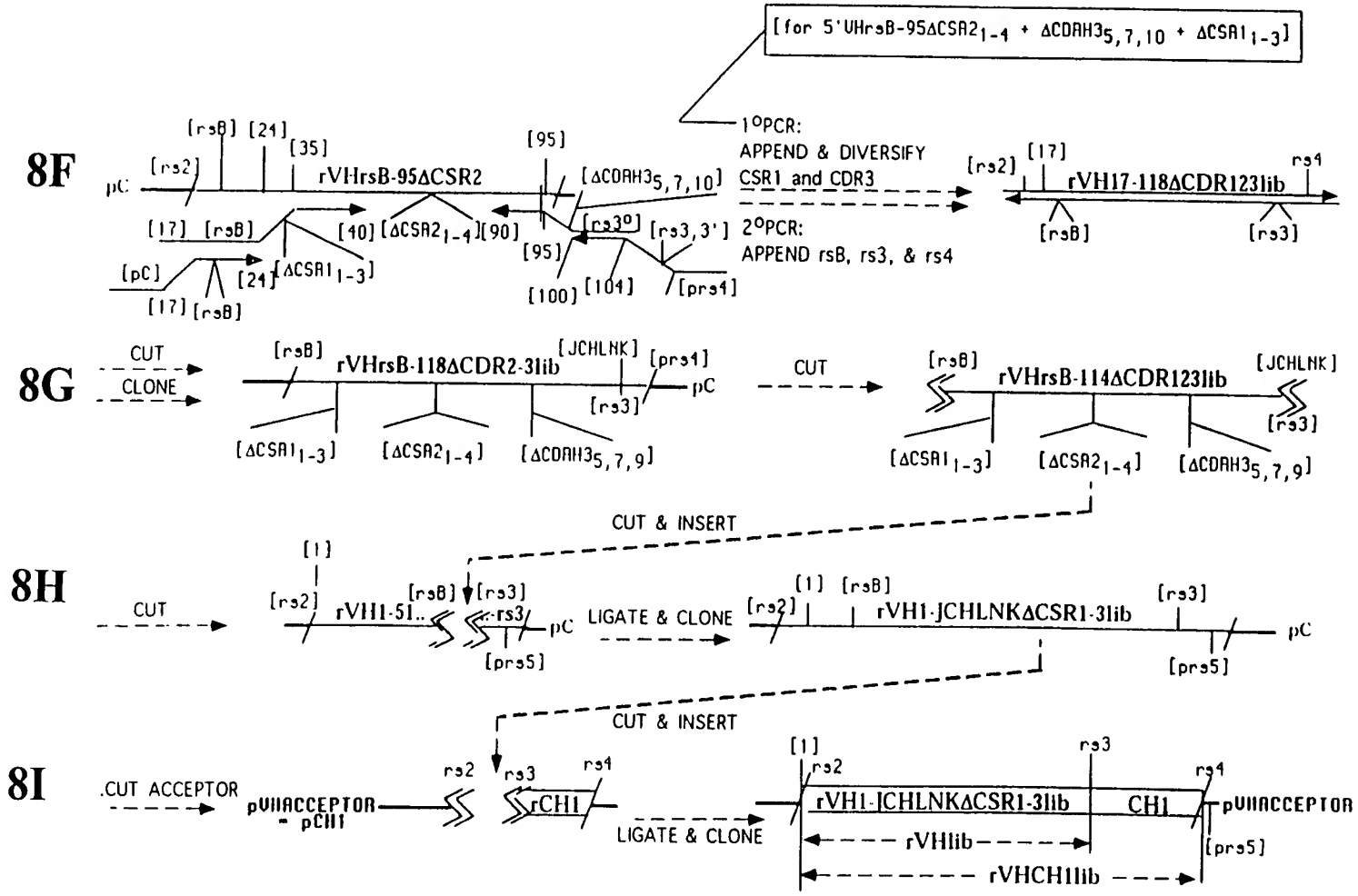
7F


[rs2']
[prs2]
rVL1-76ΔCSR1&2 1b
[rsB]
[rsC]
[prs5]
pC
LIGATE
INSERT
[rs2']
[rs2]
rVL1-100ΔCSR1-3 1b
[100]
[prs5]
pC
[1]
[8]
[rs2]
[prs0]
[95]
[100]
[rsJCLLNK]
[rs4]
[110]
APPEND
AMPLIFY
CLONE
[rs2']
[rs2]
rVL1-110ΔCSR1-3 1b
(= rVL11b.)
[110]
pC
*[rs3]
[rsJCLLNK]
[rs4]
CUT

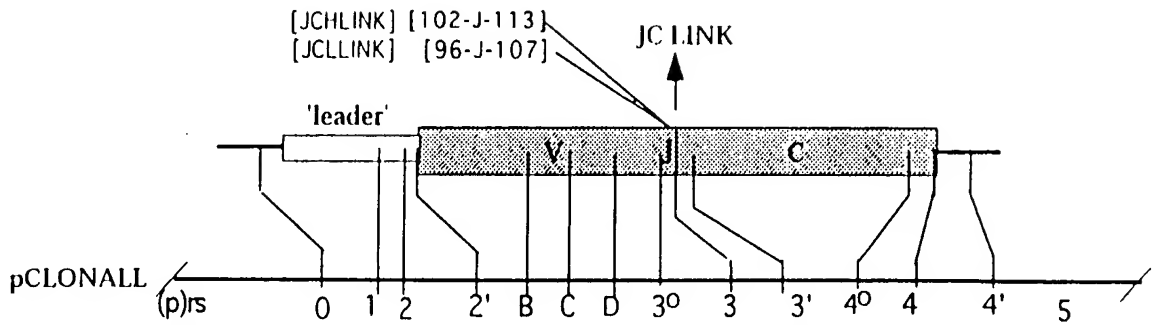
7G


[rs2]
rVL1-JCLLNKΔCSR1-3 1b
[rs3]
[rsJCLLNK]
LIGATE INSERT
CUT
[rs1]
[107]
[rs4']
CL
pVL*ACCEPTOR
[rs2]
[rs3]
*[rsJCLLNK]
[prs5]
CLONE
[rs2']
[1]
[rs3]
[107]
[rs4]
[rs4']
[rs1]
rVLΔCSR1-3 1b
CL
[rsJCLLNK]
[prs5]
pVL*ACCEPTOR
rVL11b
rVLCL11b


8A
5'VH SECTION
3'VH SECTION
[21]
[20-rsB-23]
[1]
[13][14]
[30][31]
[46]
(1)
(39)(40)
(90)(91)
(138)
a
b
c
a'
b'
c'
(1)
(30)(31)
(81)(82)
(138)
ANNEAL
LIGATE
[71*;U/A/R]
[94R]
[59]
[69][70]
[83][84]
[95]
(177)
(207)(208)
(249)(250)
(285)
d
e
f
d'
e'
f'
(177)(198)(199)
(240)(241)
(285)
8B
[1]
[rsB]
[prs2]
rVH1-46
[46]
[rs3]
[prs1]
[1] [7]
[40]
[rs4]
[47-51]
AMPLIFY
APPEND
CLONE
[(56-rsD*-59]
[71*]
[59]
rVH59-95
[95]
[prs5]
[prs1]
[59][65]
[89][95]
[pCFWD]
8C
[1] [rsB]
[20][25]
[51]
[prs1]
rVH1-51-rs3
[rs4]
pC
pC
[20][25]
[47]
[prs5]
[pC]
[52ΔCSR2$_{1-4}$56-rsD-59]
DIVERSIFY
APPEND
AMPLIFY
CLONE
[56]
[rsD*]
[71*]
[prs5]
[prs1]
rVH56-95
pC
8D
[rsB]
25
[59]
rVHrsB-59ΔCSR2lib
[prs5]
pC
[ΔCSR2$_{1-4}$][rsD]
CUT
[71*]
[rsD*]
rVH56-95
[prs5]
LIGATE INSERT
8E
[rsB]
[rsD]
rVHrsB-59ΔCSR2
lib
pC
25
[ΔCSR2$_{1-4}$]
[prs5]
CUT & CLONE
[71*]
[95]
[rsB]
[prs5]
[prs2]
rVHrsB-95ΔCSR2lib
pC
[ΔCSRH2$_{1-4}$]
[5'VHΔCSR2$_1$ + 3'VH56-95;71U]
[5'VHΔCSR2$_2$ + 3'VH56-95;71A]
[5'VHΔCSR2$_3$ + 3'VH56-95;71R]
[5'VHΔCSR2$_4$ + 3'VH56-95;71R]


8F
[for 5'VHrsB-95ΔCSR2 1-4 + ΔCDRH3 5,7,10 + ΔCSR1 1-3]
1°PCR:
APPEND & DIVERSIFY
CSR1 and CDR3
2°PCR:
APPEND rsB, rs3, & rs4
rVHrsB-95ΔCSR2
rVH17-118ΔCDR123lib
8G
CUT
CLONE
rVHrsB-118ΔCDR2-3lib
rVHrsB-114ΔCDR123lib
8H
CUT
CUT & INSERT
LIGATE & CLONE
rVH1-51..
rVH1-JCHLNKΔCSR1-3lib
8I
.CUT ACCEPTOR
pVHACCEPTOR - pCH1
rCH1
LIGATE & CLONE
rVH1-JCHLNKΔCSR1-3lib
CH1
pVHACCEPTOR
rVHlib
rVHCH1lib

9A



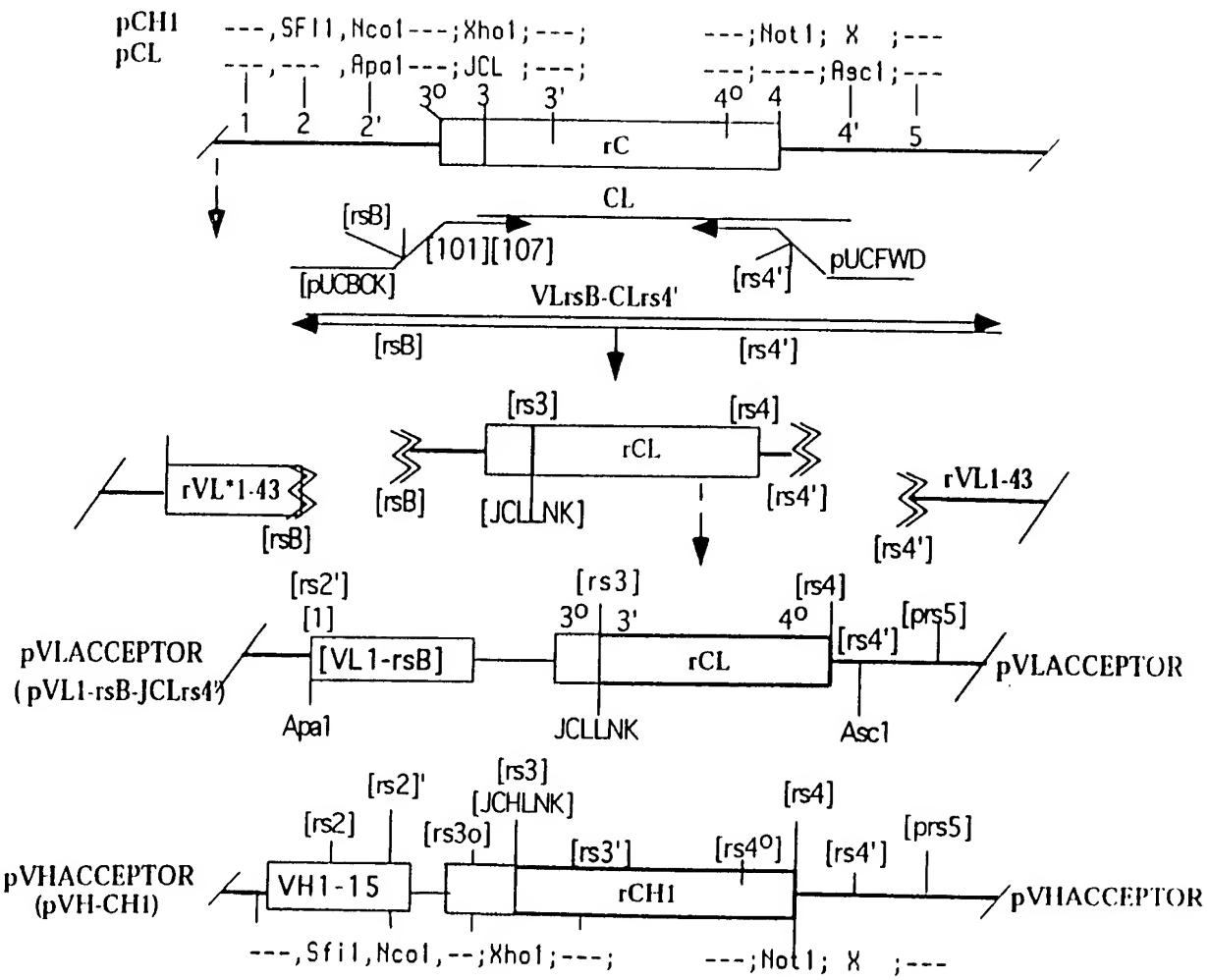
Sites used for VH constructs:-0-;Sfi1;Nco1; B, C, D ,-3°,Xho1,-3-;-4';Not1; X , --->

Sites used for VL constructs:-0-;--- ;Apa1; B, X, D ,-3°,JCL ,-3-;-4';--- ;Asc1,--->

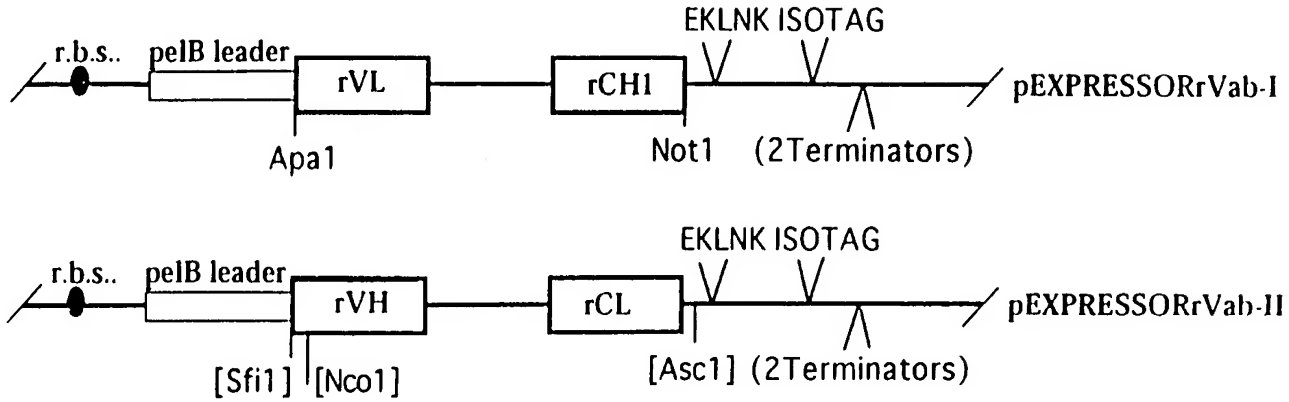
The pCLONAL cloning site:

:<---rs0;rs1;Sfi1;Nco1;Apa;B;C;D;rs3°;Xho1;JCL(rs3);rs3';rs4°;Not1;rs4';Asc1;rs5--->

9B


pCH1
pCL
---,SF11,Ncol---;Xho1;---;
---,--- ,Apa1---;JCL ;---;
---;Not1; X ;---
---;----;Asc1;---
1
2
2'
3o
3
3'
4o
4
4'
5
rC
CL
[rsB]
[101][107]
[pUCBCK]
[rs4']
pUCFWD
VLrsB-CLrs4'
[rsB]
[rs4']
[rs3]
[rs4]
rCL
rVL*1-43
[rsB]
[rsB]
[JCLLNK]
[rs4']
rVL1-43
[rs4']
[rs2']
[1]
[rs3]
3o
3'
[rs4]
4o
[prs5]
[rs4']
pVLACCEPTOR
(pVL1-rsB-JCLrs4')
[VL1-rsB]
rCL
pVLACCEPTOR
Apa1
JCLLNK
Asc1
[rs2']
[rs3]
[JCHLNK]
[rs4]
[rs2]
[rs3o]
[rs3']
[rs4o]
[rs4']
[prs5]
pVHACCEPTOR
(pVH-CH1)
VH1-15
rCH1
pVHACCEPTOR
---,Sfi1,Ncol,--;Xho1;---;
---;Not1; X ;---

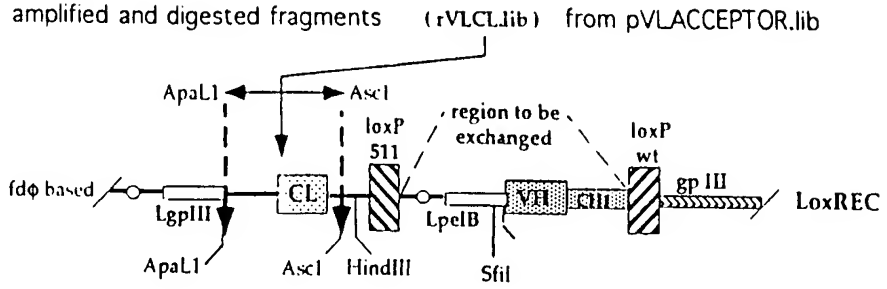
9C pEXPRESSORrVab



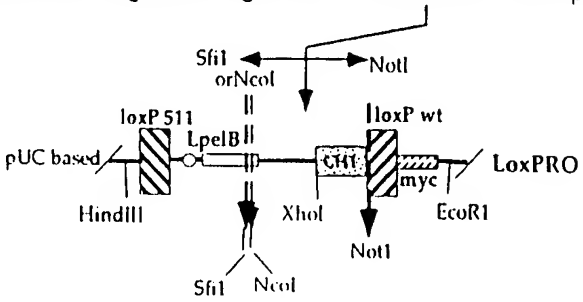
rVLlib PRIMER CONSTRUCTION				
1.	L1.1FWD:	19-24ASSSV(NNN)$_2$M3435-rsB-38-rs4.....	63mer	ΔCDR
2.	L1.2FWD:	19-24ASQSI(NNN)$_2$32L34-rsB-38-rs4.....	66mer	ΔCDR
3.	L1.3FWD:	19-24SSESL(NNN)$_2$32L34-rsB-38-rs4.....	66mer	ΔCDR
4.	L1.4FWD:	19-24SQSSL(NNN)$_2$32L34-rsB-38-rs4.....	66mer	ΔCDR
5.	L1.5FWD:	19-24GSESD(NNN)$_2$SNGNT32L34-rsX-36-rs4.....	66mer	ΔCDR
6.	L1.1-3BCK:	.....prs2-prs2',2(I)3-9	36mer	APPEND
7.	L1.4BCK:	.....prs2-prs2',2(V)3-9	36mer	APPEND
8.	L1.5BCK:	.....prs2-prs2',2(S)3-9	36mer	APPEND
9.	L1ALLFWD:	34-rsB-44-prs5.....	54mer	APPEND
10.	L1ALLBCK:	.....prs0-prs1-rs2'	45mer	AMPLIFY
11.	L271YFWK:	63-71(Y)-rsC-76-prs5(10)	49mer	APPEND
12.	L271YFWK:	63-71(F)-rsC-76-prs5(10)	49mer	APPEND
13.	L271YFWK:	63-71(A)-rsC-76-prs5(10)	49mer	APPEND
14.	L2ALLBCK:	38-rsB-48(I)49(NNN)$_2$52-58	60mer	ΔCDR
15.	L1-8ALLBCK:	.....prs1-prs2-1-8	49mer	AMPLIFY
16.	L3.1FWD:	84-89Q 91(NNN)$_2$94P9697-100	54mer	ΔCDR
17.	L3.2FWD:	84-89Q 91(NNN)$_2$P959697-100	54mer	ΔCDR
18.	L3.3FWD:	84-89Q 91(NNN)$_2$94P97-100	54mer	ΔCDR
19.	L3.4FWD:	84-899091(NNN)$_2$94959697-100	54mer	ΔCDR
20.	L3.5FWD:	84-8990919293(NNN)$_2$94959697-100	54mer	ΔCDR
21.	L3.6FWDV	84-89909192(NNN)$_2$94959697-100	54mer	ΔCDR
21.	L3ALLBCK:	prs6-72-rsC-76-82	48mer	APPEND
22.	LJCLLNKFWD:	95-100-rsC-110-rs4	51mer	APPEND
23.	CLFWD:	209-rs4'-216(rs4)-prs5	36mer	APPEND
24.	CLBCK:	prs0-105-107(CLLNK)-110-116	45mer	APPEND
rVHlib PRIMER CONSTRUCTION				
25	5'VHFWD:	40-51-rs3-pUC	54mer	APPEND
26	5'VHBCK:	prs1-1(prs2)-7	30mer	AMPLIFY
27	H1.1BCK	17-rsB-23A*25G*F*28F*30(NNN)3233M*35-40	63mer	ΔCDR
28	H1.2BCK	17-rsB-23A*25G*S*28F*30(NNN)3233M*35-40	63mer	ΔCDR
29	H1.3BCK	17-rsB-232425G*Y*28F*30(NNN)31a3233W*35-40	66mer	ΔCDR
30	H1ALLFWD	pCFWD= pCLONALLFWD (see ...)		AMPLIFY
31	H31FWD:	100-104-rs3°-rs3(CH1LNK)-rs3'-prs4	39mer	APPEND
32.	H31BCK:	pC-17-rsB-24	30mer	APPEND
33	H2.1FWD	474849505152(NNN)54G*56-rsD-59...	45mer	ΔCDR
34	H2.2FWD	474849505152P*(NNN)54G*56-rsD-59...	48mer	ΔCDR
35	H2.3FWD'	474849505152P*(NNN)G*F 56-rsD-59...	48mer	ΔCDR
36	H2ALLBCK	15-24pC	36mer	AMPLIFY
37	3'VHFWD:	89-95-rs5-pCFWD	30mer	AMPLIFY
38	3'VHBCK:	prs2-56-rsD*-59-65	39mer	AMPLIFY
39	H3.5FWD:	89-95(NNN)$_3$DY-rs3°-104	39mer	ΔCDR
40	H3.7FWD:	89-95G(NNN)Y(NNN)D(NNN)DG-rs3°-104	45mer	ΔCDR
41	H3.10FWD:	89-95Y(NNN)S(NNN)P(NNN)YFDY-rs3°-104	54mer	ΔCDR
SEQUENCING PRIMERS				
42.	pCFWD	pUCFWD = pCLONALLFWD		SEQ.
43.	pCBCK	pUCBCK = pCLONALLBCK		SEQ.

FIG. 10

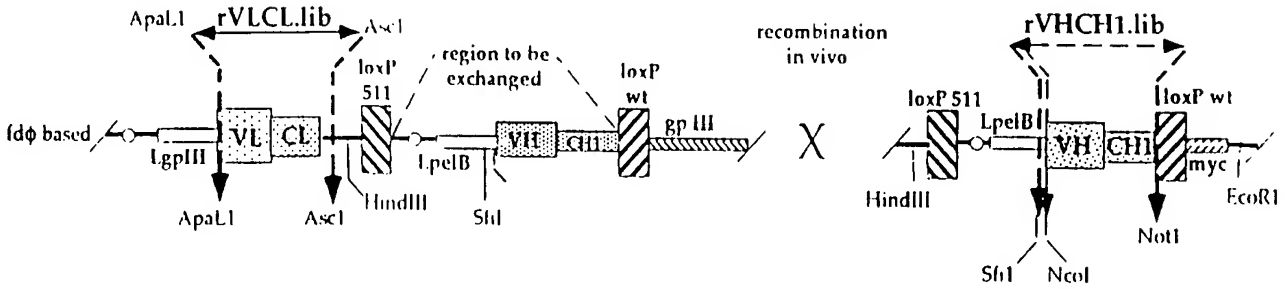
11A


amplified and digested fragments (rVLCLlib) from pVLACCEPTOR.lib
ApaL1
AscI
loxP 511
region to be exchanged
loxP wt
fdφ based
LgpIII
CL
VH
CH1
gp III
LoxREC
LpelB
ApaL1
AscI
HindIII
SfiI

11B

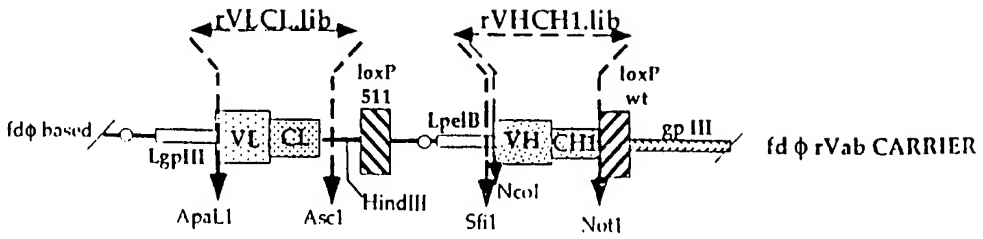

amplifed and digested fragments (rVHCH1.lib) from pVHACCEPTOR.lib
SfiI
orNcoI
NotI
loxP 511
LpelB
loxP wt
pUC based
CH1
myc
LoxPRO
HindIII
XhoI
EcoR1
NotI
SfiI
NcoI

11C Individual VHCH1 and VLCL within a bacterium are recombined in vivo (X) by Cre recombinase



LoxREC LoxPRO

11D



fd ϕ rVab CARRIER

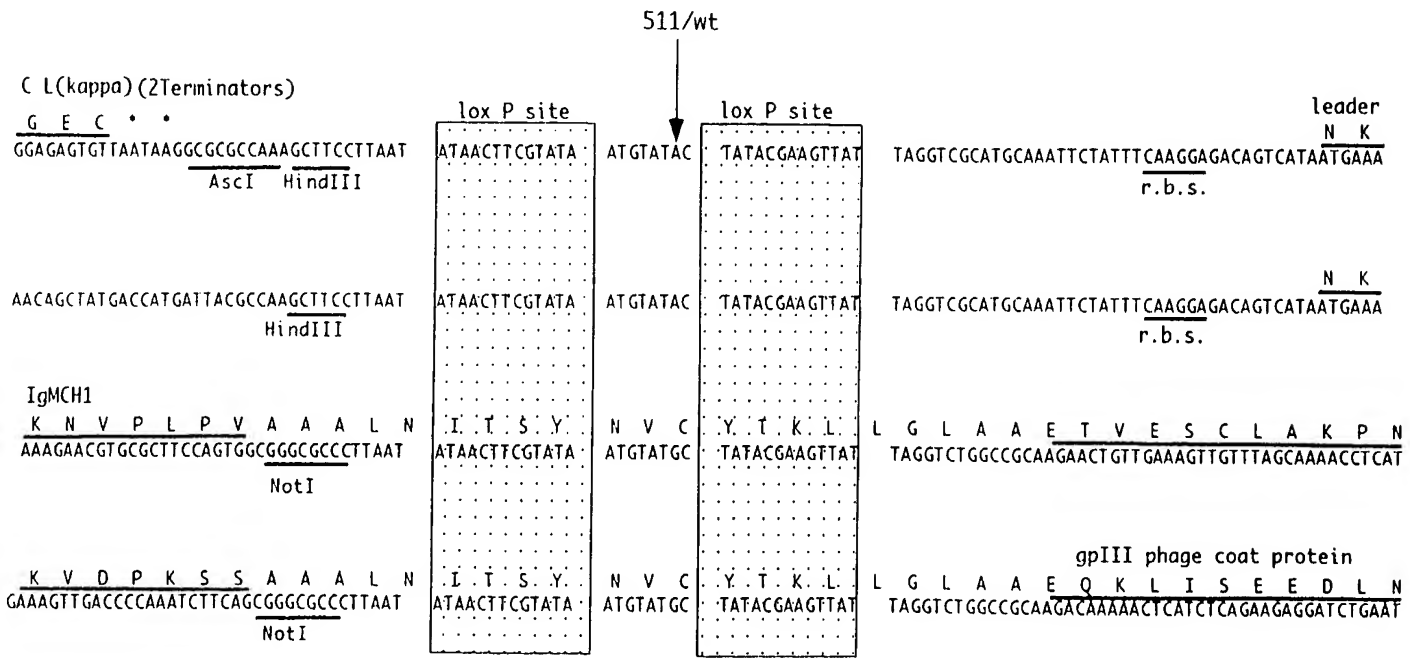
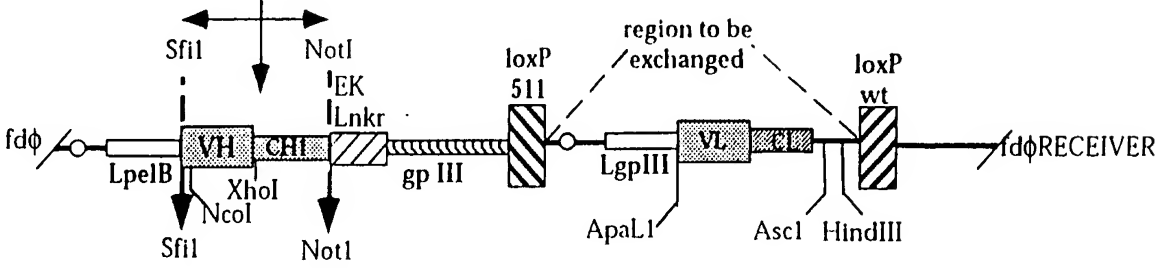
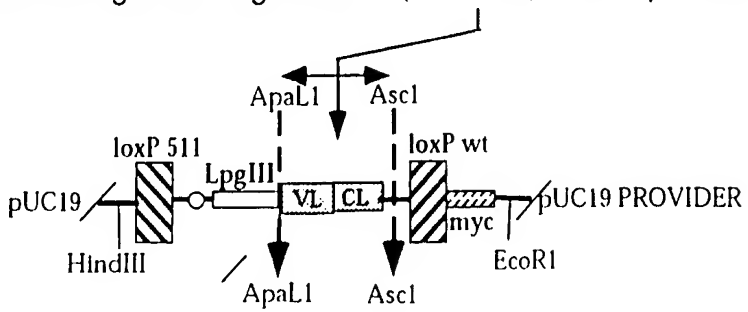

511/wt
C L(kappa) (2Terminators)
G E C • •
GGAGAGTGTTAATAAGGCGCGCCAAAGCTTCCTTAAT
AscI HindIII
lox P site
ATAACTTCGTATA
ATGTATAC
lox P site
TATACGAAGTTAT
leader
N K
TAGGTCGCATGCAAATTCTATTTCAAGGAGACAGTCATAATGAAA
r.b.s.
AACAGCTATGACCATGATTACGCCAAGCTTCCTTAAT
HindIII
ATAACTTCGTATA
ATGTATAC
TATACGAAGTTAT
N K
TAGGTCGCATGCAAATTCTATTTCAAGGAGACAGTCATAATGAAA
r.b.s.
IgMCH1
K N V P L P V A A A A L N
AAAGAACGTGCGCTTCCAGTGGCGGGCGCCCTTAAT
NotI
I T S Y
ATAACTTCGTATA
N V C
ATGTATGC
Y T K L
TATACGAAGTTAT
L G L A A E T V E S C L A K P N
TAGGTCTGGCCGCAAGAACTGTTGAAAGTTGTTTAGCAAAACCTCAT
gpIII phage coat protein
K V D P K S S A A A L N
GAAAGTTGACCCCAAATCTTCAGCGGGCGCCCTTAAT
NotI
I T S Y
ATAACTTCGTATA
N V C
ATGTATGC
Y T K L
TATACGAAGTTAT
L G L A A E Q K L I S E E D L N
TAGGTCTGGCCGCAAGACAAAAACTCATCTCAGAAGAGGATCTGAAT

FIG. 12

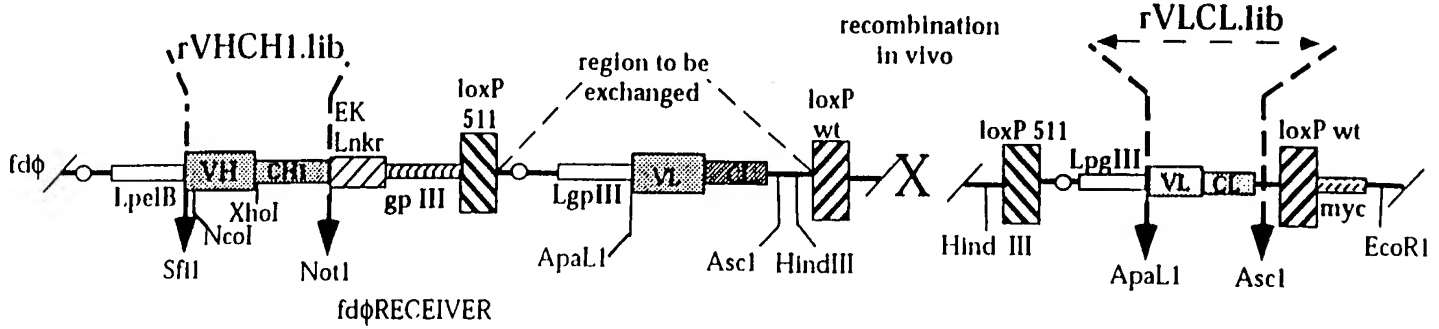
13A amplified and digested fragments (rVHCH1.lib) from pVHACCEPTOR.lib



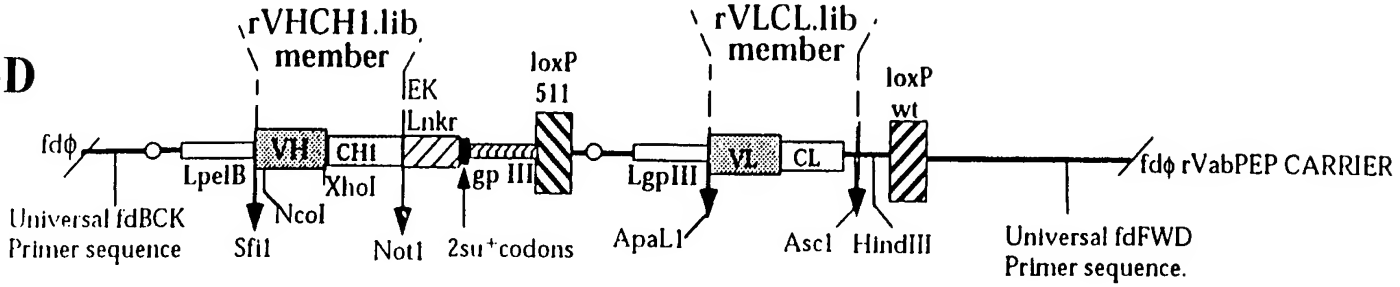
13B amplifed and digested fragments (rVLCL.lib) from pVLACCEPTOR.lib



13C Individual VHCH1 and VLCL within a bacterium are recombined in vivo (X) by Cre recombinase



13D



13E

i. Making rVab-PEP[1] Lib with Pep8 attached to Amino Terminus of VH use FWD primer Universal fdFWD and VHLKRPEPBCK primer

ii. Making rVab-PEP[1] Lib with Pep8 attached to Carboxy Terminus of CL use FWD primer CLLKRPEPFWD and Universal fdBCK primer

iii. Making rVab-PPEP[2] Lib with a Pep8 attached to Amino Terminus of VH and to the Carbocy Terminus of CL use FWD primer CLLKRPEPFWD and BCK primer VHLKRPEPBCK

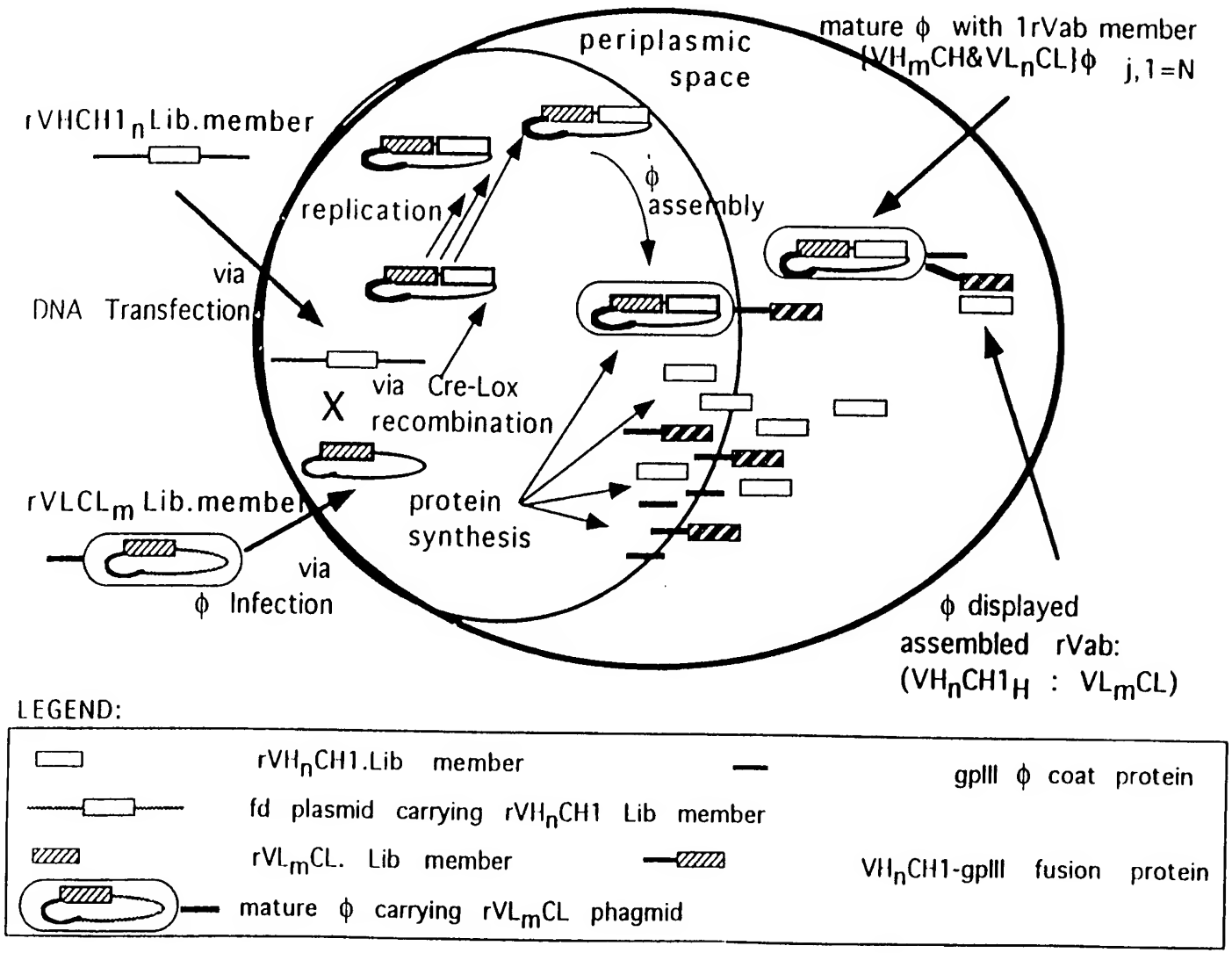

periplasmic space
mature ϕ with 1rVab member
{VHmCH&VLnCL}ϕ j,1=N
rVHCH1n Lib.member
replication
ϕ assembly
via DNA Transfection
via Cre-Lox recombination
X
rVLCLm Lib.member
protein synthesis
via ϕ Infection
ϕ displayed assembled rVab:
(VHnCH1H : VLmCL)
LEGEND:
rVHnCH1.Lib member
gpIII ϕ coat protein
fd plasmid carrying rVHnCH1 Lib member
rVLmCL. Lib member
VHnCH1-gpIII fusion protein
mature ϕ carrying rVLmCL phagmid

FIG. 14

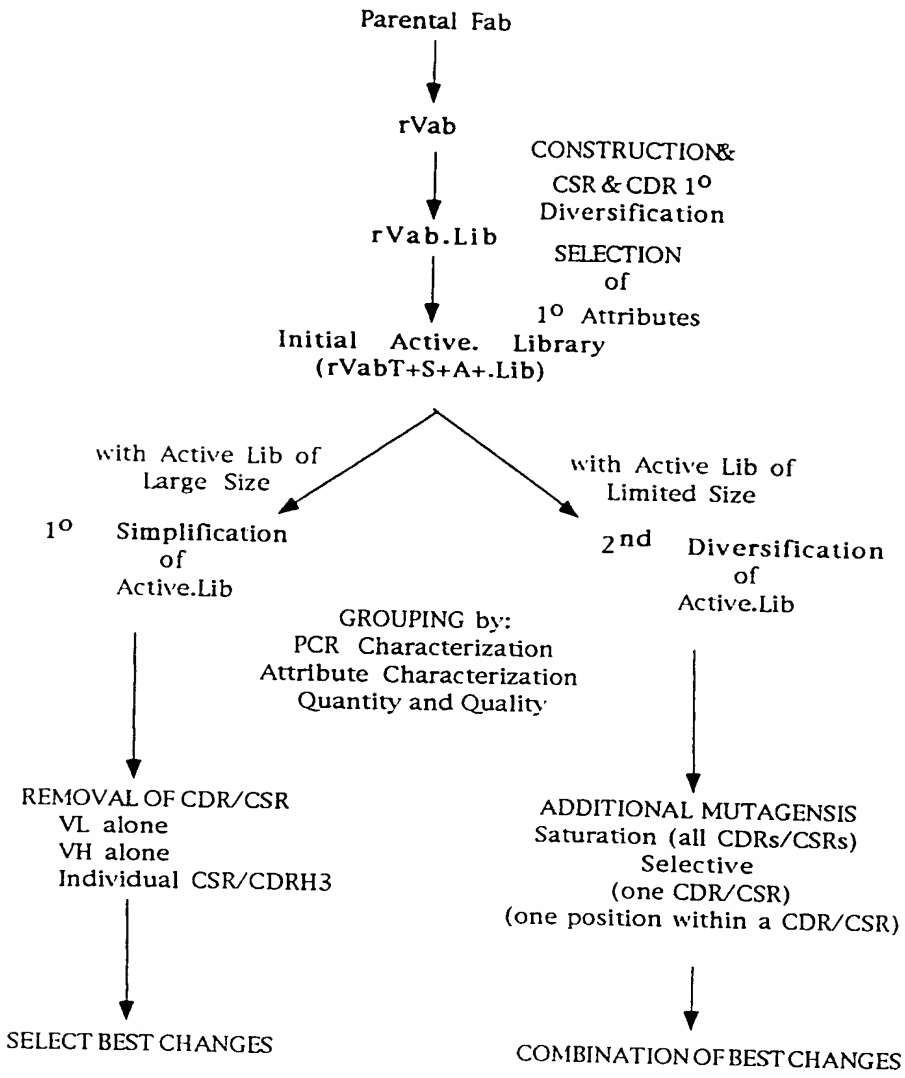
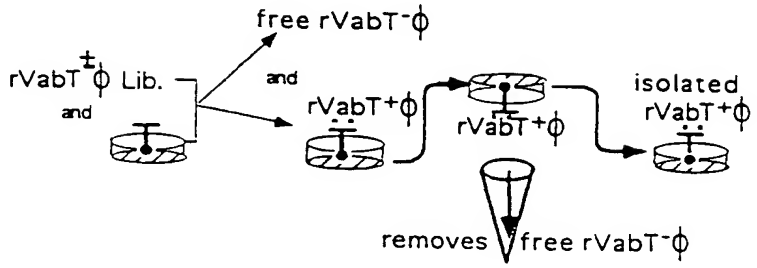
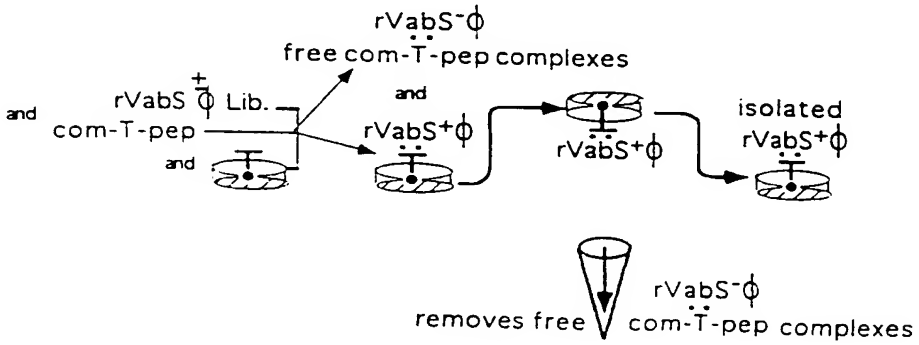

Parental Fab
rVab
rVab.Lib
CONSTRUCTION&
CSR & CDR 1°
Diversification
SELECTION
of
1° Attributes
Initial Active. Library
(rVabT+S+A+.Lib)
with Active Lib of
Large Size
1° Simplification
of
Active.Lib
with Active Lib of
Limited Size
2nd Diversification
of
Active.Lib
GROUPING by:
PCR Characterization
Attribute Characterization
Quantity and Quality
REMOVAL OF CDR/CSR
VL alone
VH alone
Individual CSR/CDRH3
SELECT BEST CHANGES
ADDITIONAL MUTAGENSIS
Saturation (all CDRs/CSRs)
Selective
(one CDR/CSR)
(one position within a CDR/CSR)
COMBINATION OF BEST CHANGES

FIG. 15

16A. Isolation for Target Recognition (T^+)



16B. Isolation for Target Specificity and/or Selectivity (S^+)



LEGEND: rVabϕ = phage displayed rVab; Lib = library;
= Target (T) bound to matrix (here plastic dishes)
com-T-pep = none-Target entity (here peptide) with undesired common surface epitopes

FIG. 16

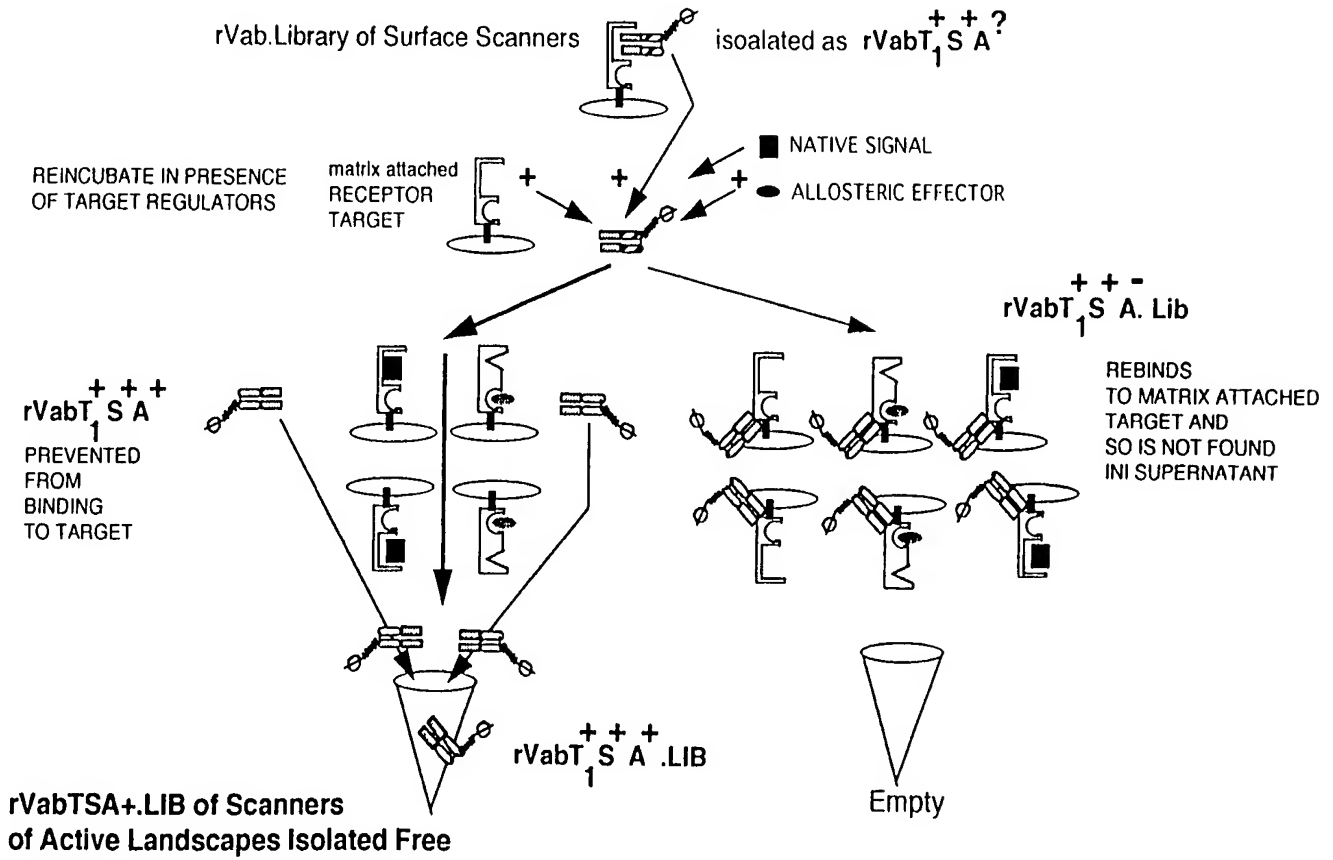

rVab.Library of Surface Scanners
isoalated as rVabT1S+A+?
NATIVE SIGNAL
ALLOSTERIC EFFECTOR
REINCUBATE IN PRESENCE OF TARGET REGULATORS
matrix attached RECEPTOR TARGET
rVabT1S+A+ PREVENTED FROM BINDING TO TARGET
rVabT1S+A- A. Lib
REBINDS TO MATRIX ATTACHED TARGET AND SO IS NOT FOUND INI SUPERNATANT
rVabT1S+A+ .LIB
Empty
rVabTSA+.LIB of Scanners of Active Landscapes Isolated Free

FIG. 17

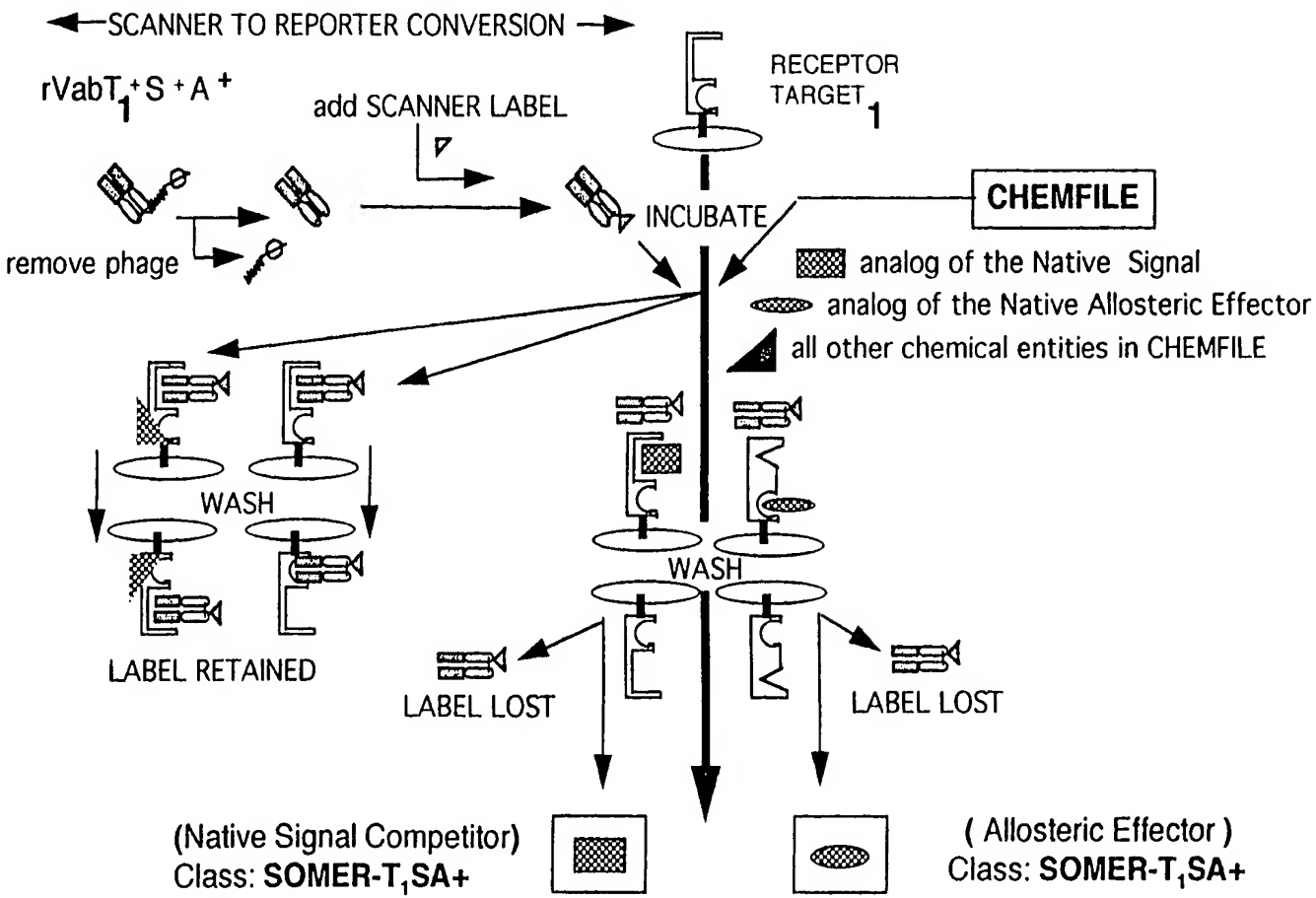

SCANNER TO REPORTER CONVERSION
rVabT1+S+A+
add SCANNER LABEL
RECEPTOR TARGET 1
remove phage
INCUBATE
CHEMFILE
analog of the Native Signal
analog of the Native Allosteric Effector
all other chemical entities in CHEMFILE
WASH
WASH
LABEL RETAINED
LABEL LOST
LABEL LOST
(Native Signal Competitor)
Class: SOMER-T1SA+
(Allosteric Effector)
Class: SOMER-T1SA+

FIG. 18

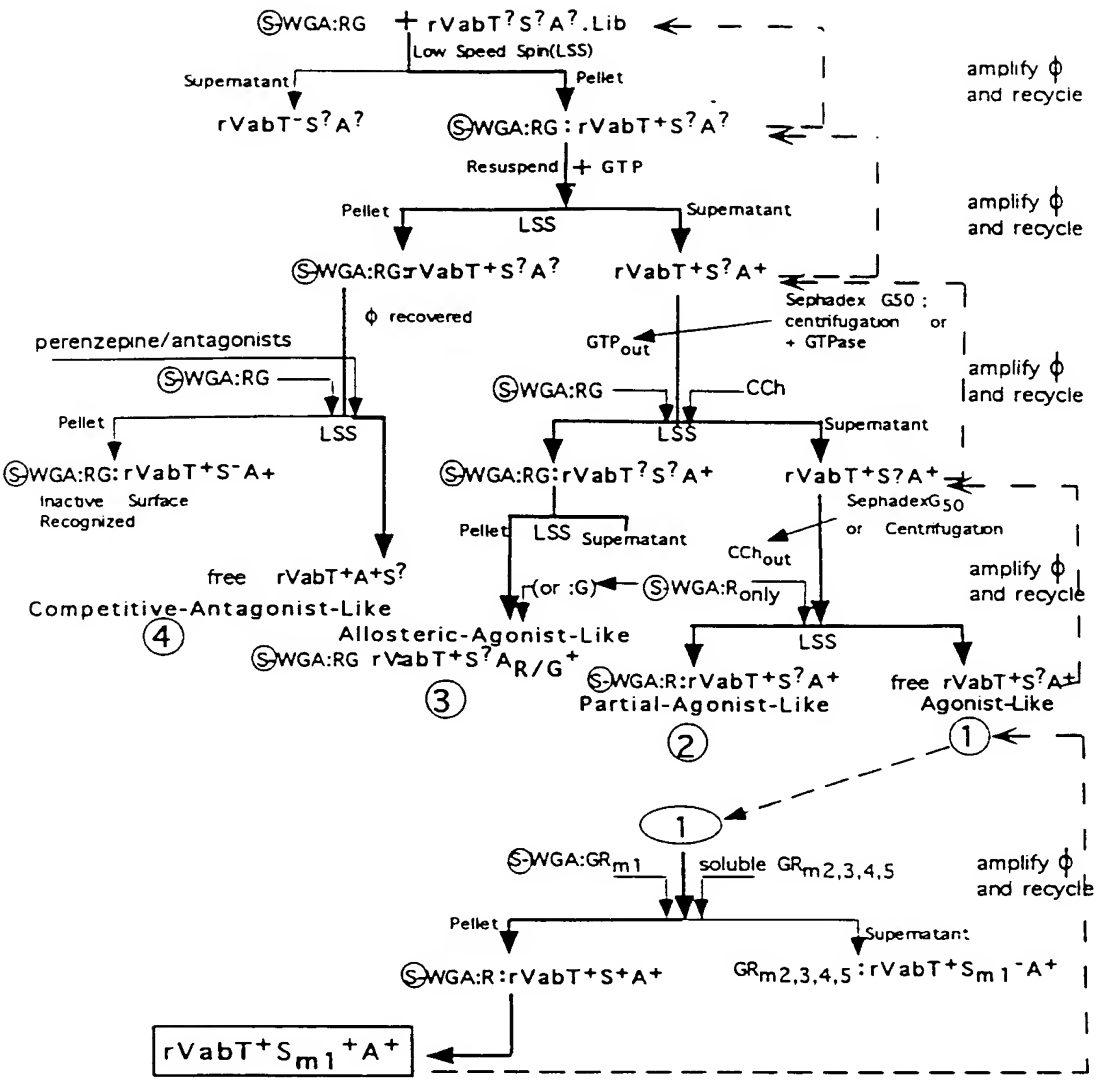

S-WGA:RG + rVabT?S?A?.Lib
Low Speed Spin(LSS)
Supernatant
Pellet
rVabT⁻S?A?
S-WGA:RG : rVabT⁺S?A?
amplify ϕ and recycle
Resuspend + GTP
Pellet
LSS
Supernatant
amplify ϕ and recycle
S-WGA:RG:rVabT⁺S?A?
rVabT⁺S?A⁺
Sephadex G50 : centrifugation or + GTPase
ϕ recovered
GTP out
perenzepine/antagonists
S-WGA:RG
S-WGA:RG
CCh
amplify ϕ and recycle
Pellet
LSS
LSS
Supernatant
S-WGA:RG:rVabT⁺S⁻A+
Inactive Surface Recognized
S-WGA:RG:rVabT?S?A+
rVabT⁺S?A⁺
Sephadex G50 or Centrifugation
Pellet
LSS
Supernatant
CCh out
free rVabT⁺A⁺S?
Competitive-Antagonist-Like
4
(or :G)
S-WGA:R only
amplify ϕ and recycle
Allosteric-Agonist-Like
S-WGA:RG rVabT⁺S?A R/G⁺
3
LSS
S-WGA:R:rVabT⁺S?A⁺
Partial-Agonist-Like
2
free rVabT⁺S?A±
Agonist-Like
1
1
S-WGA:GRm1
soluble GRm2,3,4,5
amplify ϕ and recycle
Pellet
Supernatant
S-WGA:R:rVabT⁺S⁺A⁺
GRm2,3,4,5 : rVabT⁺Sm1⁻A⁺
rVabT⁺Sm1⁺A⁺

FIG. 19

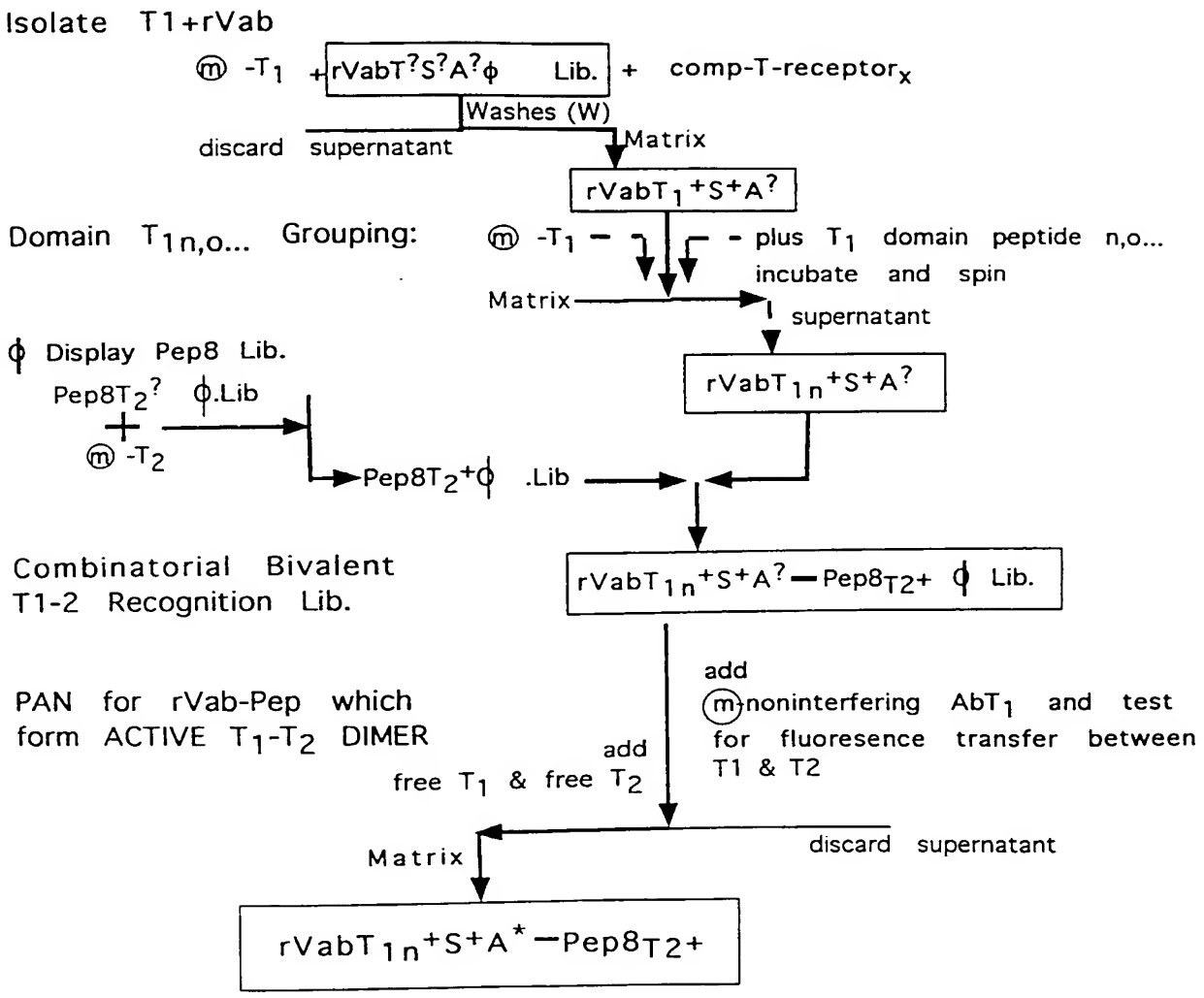

Isolate T1+rVab
ⓜ -T1 + rVabT?S?A?ϕ Lib. + comp-T-receptorX
Washes (W)
discard supernatant
Matrix
rVabT1+S+A?
Domain T1n,o... Grouping:
ⓜ -T1 – plus T1 domain peptide n,o...
incubate and spin
Matrix
supernatant
ϕ Display Pep8 Lib.
Pep8T2? ϕ.Lib
ⓜ -T2
Pep8T2+ϕ .Lib
rVabT1n+S+A?
Combinatorial Bivalent
T1-2 Recognition Lib.
rVabT1n+S+A? – Pep8T2+ ϕ Lib.
PAN for rVab-Pep which
form ACTIVE T1-T2 DIMER
add
ⓜ noninterfering AbT1 and test
for fluoresence transfer between
T1 & T2
add
free T1 & free T2
discard supernatant
Matrix
rVabT1n+S+A* – Pep8T2+

FIG. 20

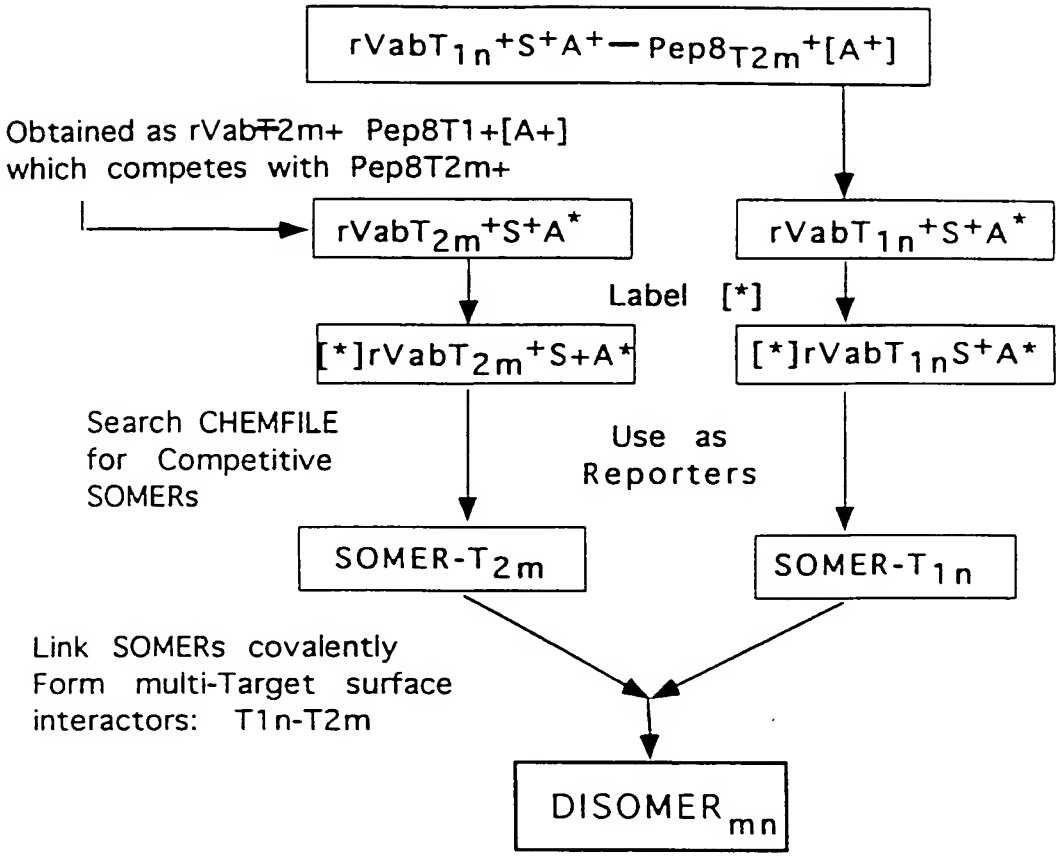

rVabT1n+S+A+— Pep8T2m+[A+]
Obtained as rVabT2m+ Pep8T1+[A+]
which competes with Pep8T2m+
rVabT2m+S+A*
rVabT1n+S+A*
Label [*]
[*]rVabT2m+S+A*
[*]rVabT1nS+A*
Search CHEMFILE
for Competitive
SOMERs
Use as
Reporters
SOMER-T2m
SOMER-T1n
Link SOMERs covalently
Form multi-Target surface
interactors: T1n-T2m
DISOMERmn

FIG. 21

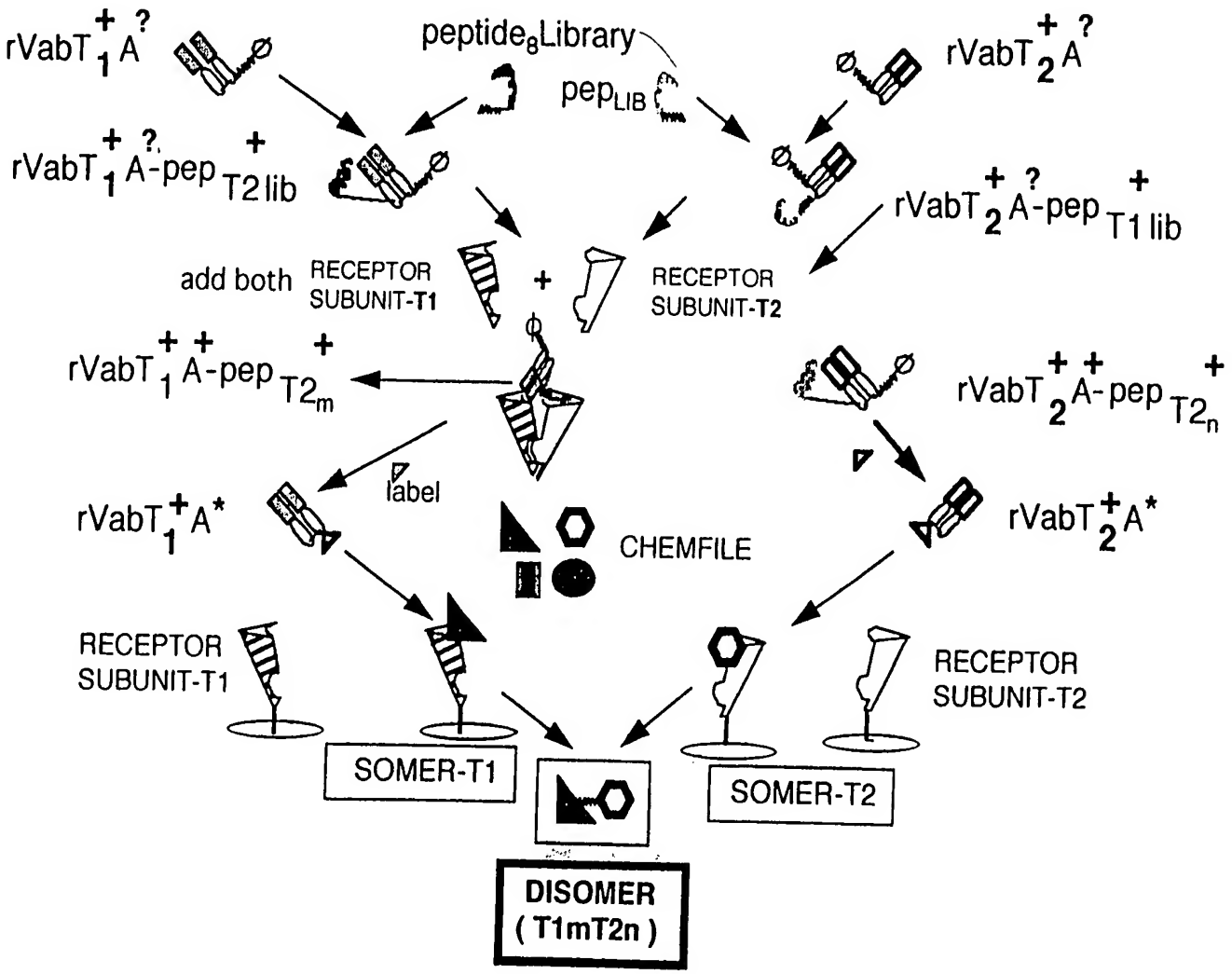

rVabT1+A?
peptide8Library
pepLIB
rVabT2+A?
rVabT1+A?-pep+T2lib
rVabT2+A?-pep+T1lib
add both RECEPTOR SUBUNIT-T1
+
RECEPTOR SUBUNIT-T2
rVabT1++A-pep+T2m
rVabT2++A-pep+T2n
label
rVabT1+A*
CHEMFILE
rVabT2+A*
RECEPTOR SUBUNIT-T1
RECEPTOR SUBUNIT-T2
SOMER-T1
SOMER-T2
DISOMER (T1mT2n)

FIG. 22

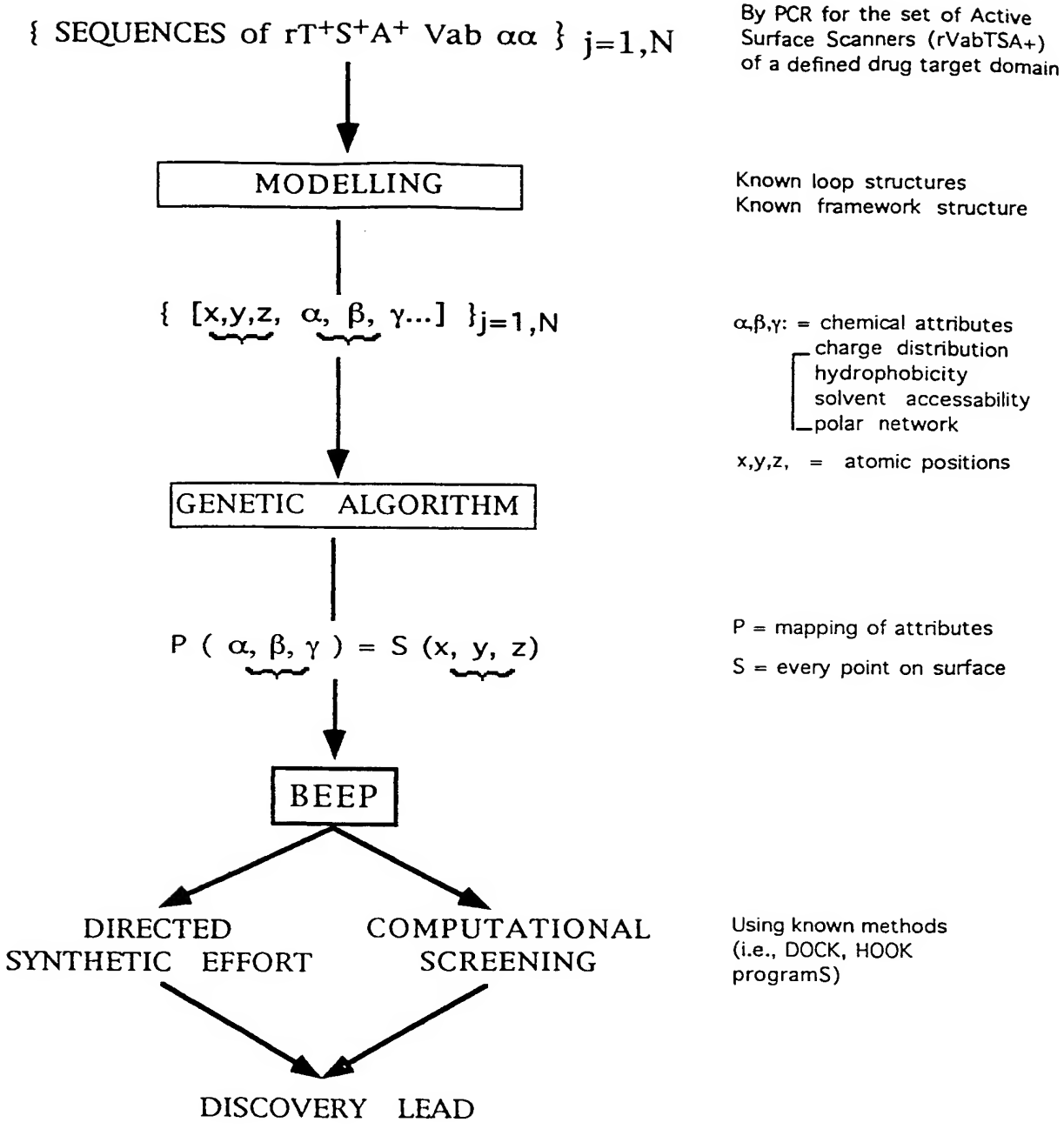

{ SEQUENCES of rT+S+A+ Vab αα } j=1,N
By PCR for the set of Active Surface Scanners (rVabTSA+) of a defined drug target domain
MODELLING
Known loop structures
Known framework structure
{ [x,y,z, α, β, γ...] }j=1,N
α,β,γ: = chemical attributes
charge distribution
hydrophobicity
solvent accessability
polar network
x,y,z, = atomic positions
GENETIC ALGORITHM
P (α, β, γ) = S (x, y, z)
P = mapping of attributes
S = every point on surface
BEEP
DIRECTED SYNTHETIC EFFORT
COMPUTATIONAL SCREENING
Using known methods (i.e., DOCK, HOOK programS)
DISCOVERY LEAD

FIG. 23

24.1 For a set of TSA+ rVabs for a specific Target:Determine the General Orientational Matrix for each attribute R_j

R = Generalized orientational matrix [ϕ, Ψ, ω] mapping similar attributes

α, β, γ = Chemical and structural attributes

$$[x,y,z, \alpha, \beta, \gamma,..]_j \longrightarrow [x^+, y^+, z^+, \alpha, \beta, \gamma,..]_j$$

$$x^+, y^+, z^+ = R_j (\phi, \Psi, \omega) (x, y, z)$$

24.2 Find the set of Rj's that minimizes some target function of α, β, γ without atomic clashes

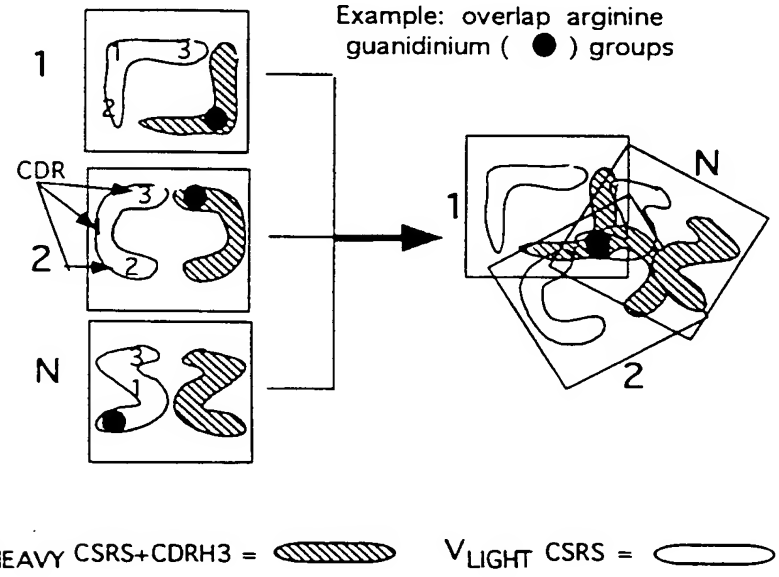


FIG. 24

1. After obtaining the first $\{attribute\}_j$; i.e., $\{ R \}_j$,
   Repeat process for hydrophobicity ; i.e., $\{ H \}_j$
   Search for the overlap of the
   $\{H\}_j$ of methyl groups with the $\{R\}_j$ of arginines

2. Now use $\{R\}_j \otimes \{H\}_j$ as good predictor of other overlaps for the other sets of chemical attributes

3. Iterate process; eliminate 'outliers' and derive a single, overlapping neighborhood Active Surface Scanner surface
   $S = \{R \otimes H \otimes \ldots\ldots Z \}_{j=1,N}$
   this is the BEEP ,
   i.e., the Biological Enhanced Ensemble Pharmacophore

4. Model of a 2D-BEEP

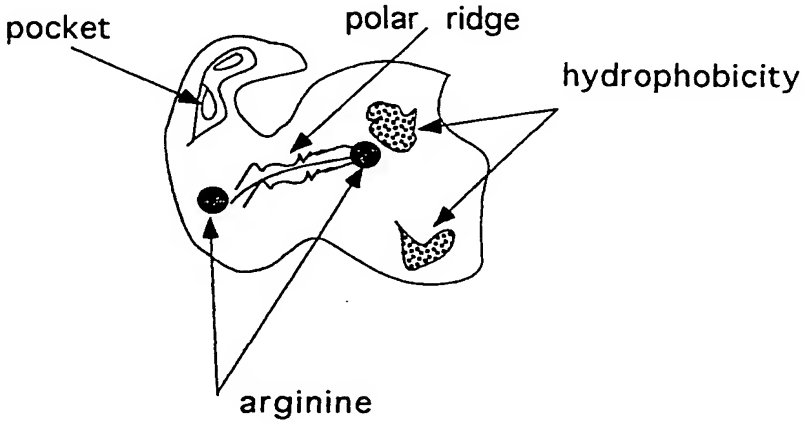


FIG. 25